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REMINISCENCES OF GERMAN BOTANICAL LABORATORIES IN THE 'SEVENTIES AND 'EIGHTIES OF THE LAST CENTURY

It is common knowledge that a very large part of the impetus to development of botany which marked the second half of the last century came from Germany, where by far the greater part of the microscopic and physiological work which laid the foundations of our knowledge of the development, structure and processes of plants was carried out. Towards the end of the 'seventies and during the 'eighties of the last century several young English botanists, inspired by the new interest given to biology by Darwin's work, and attracted by the mass of new facts and laws the Germans were revealing, sought to learn at first hand something of the methods of the great German masters of the subject. The Editor of this journal, feeling sure that the experiences of these men would be of great interest to the present generation of British botanists, has persuaded some of them to publish their reminiscences of this inspiring period of their lives.

I.

By S. H. VINES, M.A., D.Sc., F.R.S.

Sometime Fellow and Tutor of Christ's College, Cambridge, Reader in Botany in the University; later Fellow of Magdalen College, Oxford, and Sherardian Professor of Botany in the University

IT was in 1877 that I gained my first experience of botanical study in Germany. In 1876 I had been appointed an Assistant Tutor at Christ's College, Cambridge, for the benefit of the increasing number of the undergraduates who were reading Natural Science, and had begun to lecture there on Botany, though I had no means for carrying on any practical work. My experience, and I may add my practical knowledge, were limited to what I had acquired when assisting Mr (now Sir William) Thiselton-Dyer in the courses of instruction conducted by him at the Royal College of Science, South Kensington, in 1875 and 1876. It seemed to me, therefore, essential to equip myself further for future work by spending what time I could spare in some well-known laboratory. As such equipment was

quite unobtainable in Britain, my thoughts turned naturally to Germany. Then arose the question as to which laboratory I should visit. At that time the best known of the German botanists, other than systematists, was Hofmeister, though his doctrine of "alteration of generations" was still regarded with some suspicion by those of the more orthodox British botanists who knew anything about it; had he been still at work, I should probably have gone to him at Tübingen, but he had quite recently died. Considering that the greater part of my botanical lore had been derived from his immortal *Lehrbuch* (3rd ed. 1874), I had no hesitation in deciding that I must go to Julius Sachs at Würzburg, who was then at the zenith of his activity and fame, and whose laboratory was renowned for its physiological work. Having obtained leave of absence for the Easter Term from the College authorities, and armed with an introduction from Mr Thiselton-Dyer, I accordingly set out for Würzburg in March, 1877.

As soon as possible after my arrival I presented myself at the "Botanisches Institut," and was most kindly received by the Professor, the "Hofrath" as he was usually addressed, who showed me round the Institute and allotted to me a table in the laboratory.

The Institute was a lofty building of no architectural pretensions in a narrow street, standing at one corner of the Botanic Garden newly laid out on what had been part of the glacis and moat of the old fortifications of the town. The ground-floor was occupied by a medical clinic of some kind, and by the caretaker: the two upper floors and, I think, some attics were appropriated to Botany. On the first floor was the lecture-room, a large but rather low room capable of accommodating about 80-100 auditors; and two or three small rooms for the Professor, one of which contained the modest Herbarium of the University and the few books that constituted the library. On the second floor, over the lecture-room, was the laboratory reserved for the advanced students: adjoining this was a small passage-room occupied by the "Assistent," leading to a long narrow room, facing the Garden, which served as a museum and as a class-room for the practical work of the elementary students. There was also another room, across the passage and facing the street, in which the physiological apparatus was kept, and which could be used for experiments.

The accommodation afforded by the Institute was thus distinctly limited, according to modern ideas. So too was the Staff, which consisted only of the Professor, the Assistent and the caretaker.

As I arrived in the Easter vacation, it was not until the "Sommer-Semester" opened that I was able to form an idea of the normal course of the work carried on, which was as follows. The Professor gave a course of lectures on general Botany to a fairly large audience consisting chiefly of medical students, without any accompanying practical work, so that after his daily 8 o'clock lecture was over he had the rest of the day free for his other occupations, the supervision of the work of the advanced students, his own researches, and the administration of the Institute and of the Garden. The elementary practical course, the "Mikroskopisches Praktikum" as it was termed, was held for a couple of hours once or twice a week, and was attended by the few ordinary students who were sufficiently interested in the subject to devote this extra time to it. It was conducted by the Assistent—then Dr Detlefsen—whose only other duty was to assist the Professor in his own work when called upon to do so, which was not very often so far as I could observe. In fact the idea of the post seemed to be that, in return for the discharge of these light duties, the holder of it should enjoy the privilege of using the laboratory for his own research, with a remuneration so meagre that I do not venture to mention it.

Clearly teaching was altogether secondary to research. There was none of that laborious and elaborate practical instruction for all the students which we in England have held to be essential, and which lays such a heavy tax upon the time and energies of our teachers. Naturally the German examinations did not include any practical work: in fact they were entirely *viva voce*. I could not help feeling a certain satisfaction that here in Germany there was nothing to approach our pioneer courses at South Kensington as far as practical teaching went.

On beginning work in the laboratory, I found myself to be the only advanced student, so that I had the great advantage of the undivided attention of the Professor, which, I gratefully acknowledge, was ungrudgingly bestowed. The special subject of study suggested to me was that of growth, especially in its relation to light, and Sachs placed at my disposal and demonstrated to me all the available apparatus for measurement. Such was my first introduction to practical plant-physiology. The routine involved daily attendance, beginning not later than 8 a.m., often earlier, for the laboratory opened at 6 a.m.; an interval for dinner, from 1-2 p.m., or sometimes a little longer when the weather was very hot; and then work again till about 6 p.m., when the laboratory closed.

Naturally I saw a good deal of the Professor in those early days, and the acquaintanceship developed into an intimacy which continued unbroken until his death in 1897. I often accompanied him for a stroll round the Botanic Garden or in the shady avenues of the town, when he won my admiration by his remarkable conversational gift, discoursing of many things, not always botanical by any means, but ranging widely to include such topics as the philosophy of Herbert Spencer or the works of Lecky. Though Sachs could read English easily and understood it fairly well when spoken, he could not speak it at all fluently, so that our conversation was mostly in German: but sometimes he would ask me to speak to him in English whilst he spoke in German. He was then engaged upon his experiments, by the lithium-method, on the rate of the transpiration-current, in which I occasionally helped him and had the opportunity of observing the prevision and skill with which he devised and carried them out.

As for the man himself, in his habit as he lived, Sachs was at that time in the full vigour of his 45 years, a sturdy figure of middle height, looking more like an artist than a Professor. His rubicund face, with its reddish moustache and closely clipped pointed beard, was surmounted by a shock of darker hair brushed back from his rather prominent forehead, and his eyes sparkled with humour. Though his manner was naturally genial, yet when necessary he could be stern, and would then express himself in forcible language.

With the opening of the "Sommer-Semester," I had the opportunity of attending the Professor's lectures. Elementary though they were, he delivered them with such lucidity and force that the familiar things became instinct with new life. His only rival as a lecturer, in my experience, has been Professor Huxley.

The rather solitary life in the laboratory was enlivened for me, in the latter part of the Semester, by two notable events. The first was the arrival from Cambridge of Professor Bower, then, I think, still an undergraduate of Trinity, who followed my example in coming to Würzburg. The second was the advent of Dr Ernest Stahl (afterwards Professor in Jena), bringing with him all the glamour of his recent discovery at Strassburg of the carpogonium of the Lichens, who came to "habilitate" himself as "Privat-Dozent" in Botany. Professor Bower and I had the privilege, shortly afterwards, of attending his "Habilitation" in the Aula of the University, in the imposing presence of the Rektor Magnus and of many of the Professors in academic robes, their "Ornat." We could not but pity

Dr Stahl as, on that torrid afternoon in July, in full evening dress, he lectured on his "*Habilitations-schrift*," and then defended his Theses against three or four "*Opponenten*," by the space of two hours. Needless to say that, though much exhausted, he successfully withstood the ordeal. The Semester quickly drifted on to its end, and shortly afterwards I left Würzburg. I had come but little into contact with the general life of the University, not at all, in fact, with the student-life, and only to a limited extent with the Professoriate: it was, however, a rare privilege to have consorted not only with Sachs, but also with such eminent biologists as Karl Semper, the Professor of Zoology, and Albert Kölliker, the Professor of Anatomy.

During the two following years I carried on botanical teaching at Cambridge, now including practical laboratory work, thanks to the kindness of Sir Michael Foster who lent me a room for the purpose in the then newly erected Physiological Laboratory. At length more permanent accommodation was provided by Professor Babington, who assigned to me a couple of ground-floor rooms in what was then the Botanical Department, now occupied by the Department of Mineralogy. I may mention incidentally that it was necessary for me to provide the requisite apparatus, including a dozen Zeiss microscopes, some of which are, I believe, still doing service in the Botanical Laboratory.

The wholly amicable arrangement with Professor Babington stipulated that my activities should be limited to what was then termed "*Physiological Botany*," whilst the Professor reserved the domain of Systematic Botany to himself. Even with this limitation I found it difficult to do justice to all the various branches of the subject that I had to teach; and continually felt the need for more study on my own account than I could possibly secure in the intervals of the ever-growing teaching work. The most crying need was perhaps that of an acquaintance with the methods for the study of the Fungi, and there was no better means of meeting it than a visit to Professor de Bary's laboratory at Strassburg. So at the beginning of the Long Vacation of 1879 I made my way there, with five or six weeks for work. The Professor made me welcome and assigned to me a table in the laboratory. In appearance he was entirely different from Sachs; a slight figure, with a pallid rather ascetic face wearing a grey beard; but for all that he was vivacious and humorous as well. He was most kind and helpful, putting me through various elementary methods such as hanging-drop culture, etc. The few weeks passed pleasantly but all too quickly away.

This short stay sufficed only to make me wish for a more prolonged course of study. I succeeded in obtaining leave of absence from Christ's College for the Lent and Easter terms of 1880, and I travelled back to Strassburg in January of that year. On resuming my place in the laboratory, I found it well-filled. Professor Bower was there and others who have since become distinguished. Of these others I recall Zacharias, afterwards Professor in Hamburg; Klebs, afterwards Professor in Basel and later in Halle; Schimper, who went to America as Professor in the Johns Hopkins University, Baltimore, and then returned to be a colleague of Strasburger in Bonn; Büsgen, who also became a Professor somewhere in Germany; and Leo Errera, then engaged in his well-known work on the presence of glycogen in yeast, afterwards Professor in Brussels, presiding over the laboratory which he caused to be built.

Here again I had but little contact with the University in general. It strikes me as strange that there should have been but little intercourse even among ourselves outside the laboratory. The only one of those working there whom I came to know at all intimately was Errera, a singularly genial and accomplished man. We remained in correspondence until his sudden and lamented death in 1905, and we met several times as the years passed, once, I well remember, in his own home. The only Professor, other than de Bary, with whom I became acquainted was Hoppe-Seyler, the eminent bio-chemist, who had turned his attention to plants and was then working at chlorophyll.

But I must not omit to say something about the Botanical Institute. It was still housed in the old "Académie" building, taken over from the French, in which Millardet had worked, adjoining a small Botanic Garden. It consisted of a rather small lecture-room on the ground-floor, and, on the first floor of a fairly large room, the laboratory, with windows on three sides, together with a smaller room for the Professor. So far as I discovered there was no herbarium, library, or museum. The accommodation was thus even more limited than that in Würzburg. The staff consisted solely of the Professor and the laboratory-attendant, though I seem vaguely to recall that Dr Schimper had some teaching function to perform for some part of the year.

I must confess that my anticipations were not fulfilled on this second visit to Strassburg. The Professor suggested to me some work on the sporulation of yeast, but it led to nothing: for some reason or other the yeasts used could not be induced to produce spores.

Nor were there compensations in other directions. The Professor was lecturing, not on Fungi as I had hoped, but on plant-anatomy in relation, no doubt, to his *Vergleichende Anatomie* which had been published two or three years before. The lectures were delivered in the small rather over-heated lecture-room at 5 p.m. by dim gas-light; and though de Bary was a lucid lecturer, his delivery was rather monotonous, so that the general effect was not inspiring. As I did not feel that I was making the best use of my time, I decided, perhaps rather precipitately, to limit my stay at Strassburg to about three months and to spend the remainder of my time at Würzburg.

Accordingly I returned to Würzburg in April, and found the laboratory much as I had left it three years before. But the "Assistant" was now Dr Goebel (in after years Professor of Botany at Munich), and I was not a solitary student for I had the company of Dr D. H. Scott who had spent the "Winter-Semester" in the University. A Dr Zimmermann, who afterwards became well known in connexion with cytology, also worked in the laboratory at times. Professor Sachs was as friendly and energetic as ever, though I did not see so much of him as in 1877, for he was engaged not so much in experimental work as in the preparation of his remarkable papers on "*Stoff und Form der Pflanzenorgane*" in which he propounded the theory that the specific form of the various members of the plant-body is determined in each case by the presence and action of a specific substance even though in very small proportion, a theory which has since taken more definite shape in the modern doctrine of hormones.

I did not, on this occasion, undertake any definite piece of work, but continued the investigation of the chemical composition of aleurone on which I had been engaged off and on for some time, in which the Professor took a lively interest as it was a subject to which he had not devoted much attention. I had also an opportunity of doing some chemical work on the proteins of plants in the Physiological Institute under the guidance of Dr Kunkel who was a Privat-Dozent in that Department.

But the outstanding feature of the Semester was the Professor's course of demonstration-lectures on plant-physiology. The lectures were given on the Saturday mornings, and each lasted for about two hours. Here Sachs was at his very best, inspired by enthusiasm for his subject which he did not fail to communicate to his audience. The eloquent speech; the pictorial illustration, generally by means of large sheets of white paper and a stick of charcoal instead of

black-board and chalk; the manipulative dexterity; all these combined to rivet attention. The only drawback was that, not infrequently, the morning was cold and dull, and then the experiments were not always quite successful, much to the annoyance of the lecturer. With this last and most favourable impression my "Wanderjahre" came to an end.

Such is my story, but it would be incomplete were I to conclude without some moral reflections. The first and most obvious is—how simple, we should now call them inadequate, were the means with which the great advances in botanical science, between 1840 and 1880, were achieved in Germany! Clearly great epoch-making discoveries do not depend upon huge superlatively equipped laboratories: it is the man, not the mechanism, that counts. The next takes the form of the question—was it worth while to go to Germany to study? My answer is a strong affirmative. Brief and fragmentary as were my studies there, I recognise how great was their advantage to me, and I do not forget the debt of gratitude that I owe to the Professors who so kindly received and helped me. If the enquiry be pressed further—what was it that I gained? my answer is that I gained, not so much actual knowledge as what, for lack of a better term, I must call inspiration, the right point of view; in fact a sort of botanical "confirmation" at the hands of the pontiffs of the science. To make my meaning clear, I may explain that though I had read much of what there was to read on plant physiology (it was relatively little in 1877!), I had had no opportunity of comparing notes with another plant-physiologist until I went to Sachs, the fountain-head of so much of the knowledge which I had laboriously gleaned from books. His expositions of his own work, and his criticisms on the work of others, including my own, were a liberal education. I sought and found in Germany what was unobtainable in my own country.

II.

GERMAN REMINISCENCES OF THE EARLY
'EIGHTIES

By D. H. SCOTT, M.A. (Oxon), Ph.D. (Würzburg), F.R.S.
Formerly Honorary Keeper of the Jodrell Laboratory, Royal Gardens, Kew

MY experience of University life in Germany extended, on and off, over a period of just two years and a half, from the end of February, 1880, to the end of August, 1882; it was confined to Würzburg, in Bavaria. I went there on the advice of Thiselton-Dyer, backed up with more caution by Vines, in order to work under Sachs.

At that time, Sachs had a high reputation in England, owing, in a great degree, to his famous Text-book, of which an English edition, translated by Bennett and Thiselton-Dyer, had already appeared in 1875. This had a marked influence on the progress of Botany in England.

The thought of going to Germany to do Botany had, for me, an almost romantic charm. As a boy, I had read English translations of works by Alexander Braun, Nägeli, von Mohl and Hofmeister, while the *Micrographic Dictionary*, with its constant citations of German botanical literature, helped to fire my enthusiasm. It was indeed a great band of botanists who adorned German science about the middle of the last century. Thus, going to work in a German laboratory was to me like a pilgrimage to Mecca to a pious Mussulman.

At that time, we were still in the afterglow of the Golden Age of Botany. Schleiden himself, that erratic genius who founded the cell-theory on its botanical side, only died while I was at Würzburg. Sachs, who belonged to a later phase, had, I fancy, something in common with the great master. Both were singular and brilliant personalities and both exercised a vast influence, though they often proved to be wrong in their theories.

On arriving at Würzburg I called on Sachs and had an interview with him in his own house—the only time I ever entered it. My work at the Botanisches Institut began the next day. The Institute at that time was an old-fashioned but fairly roomy building, adjoining the Botanic Garden.

The first four or five months were devoted to a general training in microscopic Botany—anatomy, cytology (to use a more modern term) and the phenomena of reproduction. Sachs gave me an excellent course. From the first day onwards, I had to draw what

I saw, as soon as I got a preparation good enough to show anything clearly. "Was man nicht gezeichnet hat, hat man nicht gesehen" ("What one has not drawn, one has not seen"), was a saying of Sachs's; he was himself a most accomplished draughtsman; he never used or advised others to use any mechanical aids such as a *camera lucida*. I never attained to much skill, but a few of my Würzburg efforts are reproduced as figures in *Structural Botany*¹. A good deal of the material was cut fresh, though, of course, spirit-specimens were much used also. The microscopes were of a simple type, usually with no coarse adjustment beyond the sliding tube. Immersion objectives were already in use, but only water-immersion, so far as I know. I never saw a microtome.

At that time Goebel was Privat-Dozent and Assistant to Sachs. I had a great regard for him from the first, both on his own account and because he was a pupil and ardent admirer of the great Hofmeister, who had long been my chief botanical hero.

Sometimes we went out for a walk and occasionally Sachs himself was of the party. Longer expeditions in those days were usually with Goebel and Darapsky, a Czech then working in the laboratory.

Now, of course, the Czecho-Slovakians are our allies. At that time I did not notice any special friendliness. I remember Darapsky exclaiming scornfully, when some British possession was mentioned, "Die ganze Welt gehört England" ("The whole world belongs to England"). The Germans themselves were generally friendly enough: in those days the idea of a war between our country and theirs would have seemed to me simply absurd.

At Würzburg I made the acquaintance of the veteran biologist Albert von Kolliker, who showed me hospitality. He was a splendid old man, handsome, a keen sportsman and gifted with a good sense of humour. He was altogether the finest type of the German man of science, and I need not say how proud one was to know personally one of the old guard, the founders of modern Biology.

There were no students, in the ordinary sense, working in the laboratory, but only one or two, like myself, who hoped to become botanists. Students (chiefly medical) attended the lectures; on rare occasions Sachs gave a demonstration in the laboratory; that was all.

Sachs's elementary lectures began, after the Easter Vacation, on April 29th. They were at 8.15 in the morning in summer ("das akademische Viertel" was always allowed, so that the ordinary lecture only lasted three quarters of an hour).

¹ Part I, Figs. 89 and 90; Part II, Fig. 54.

Sachs was the best lecturer I ever heard. Hard work at German, before starting from England, had prepared me to understand what I heard without much effort. The lectures were extraordinarily clear, interesting, and sometimes amusing. On one occasion, when Sachs was lecturing on insectivorous plants, he mentioned the objection that they were able to live without animal food. This he met by saying: "In Poland and Ireland a great many people live only on potatoes, but it does not follow that a beef-steak wouldn't be a good deal better."

Another time Sachs was criticising the loose statement often made, that a tissue "grows by cell-division." He remarked that the only case on record of growth by division was the Miracle of the loaves and fishes!

The brilliancy of Sachs's lectures was enhanced by the beauty of the sketches which he made to illustrate them. Usually these were on the blackboard, but when any specially elaborate structure was to be shown, a sheet of cartridge-paper was brought in, and Sachs made, before our eyes, a finished chalk drawing in colours—truly a wonderful performance. Later in the season Sachs started his physiology lectures, given on Saturdays. In this case, as experiments had to be demonstrated, two hours (actually one and three quarters) were allowed. Though I was never attracted to physiology, it was a great experience to witness these expositions by the greatest plant-physiologist of his time.

Like all who work with living plants, Sachs was to a certain extent at the mercy of the weather. I remember his bitter complaint during one of those cold spells which constantly recur in June: "Bei diesem ganz abnormen Wetter ist es absolut unmöglich was zu zeigen" ("In this quite abnormal weather it is absolutely impossible to show anything").

In conversation also Sachs was brilliant; often his remarks were caustic. On one occasion I had been working at *Botrydium*, which Goebel had shown me where to find in the town ditch. I was inclined to go off into the life-history of the plant, then supposed to be more elaborate than it has since proved. This digression did not meet with Sachs's approval; he recalled me to the narrow way with the words: "Ich denke Sie untersuchen die Zellkerne" ("I think you are investigating the nuclei").

The hours were long: we were supposed (as mentioned already) to attend the 8.15 lecture, and work went on (of course with a long intermission for Mittagessen) till supper time at 8 o'clock. I re

member, one day, leaving the laboratory about 6.30, I met Sachs in the garden and he asked me: "Machen Sie Feiertag, Herr Scott?" ("Are you taking a holiday?"). Often, also, we worked on Sundays.

Botanical excursions, under Goebel's leadership, began on May 8th. Our second expedition was to the Güttenberger Wald, the nearest forest to the town. This reminds me of an incident, when I first heard of the Güttenberger Wald, from the landlady of the Hotel. I asked her if it was pretty there. She replied: "Ach, ja, sehr gutes Bier!" ("Oh, yes, very good beer!").

Our most sensational find on the excursions was *Cypripedium Calceolus*, in the Edelmann's Wald, on May 29th, a wonderful sight to an English botanist, for whom the plant has an almost mythical glamour. *Vincetoxicum officinale* and *Dictamnus Fraxinella* were other remarkable specimens found that day, representing families unknown in the British flora.

Altogether the country and its plants were a great source of pleasure. *Anemone Pulsatilla*, *Euphorbia Cyparissias* and *Gagea arvensis* were common species which we seldom see at home. On the other hand, the Central German Flora was equally remarkable for its deficiencies; I never saw a Primrose or a Bluebell, nor can I recall a Furze-bush.

Primroses were represented by the Oxlip (*Primula elatior* Jacq.), and in some of the woods (though at a distance from Würzburg) Bluebells were magnificently replaced by sheets of *Anemone Hepatica*. Wünsche's *Schul-Flora* was the book we used for naming our plants, and a very good book, too.

Vines came to Würzburg in April and stayed about three months. He had previously worked both there and at Strassburg, and was an excellent German scholar. I have always looked back on our association at Würzburg with great pleasure.

About that time I made the acquaintance of Zimmermann, who became well known as a botanist; he was a Brunswicker and a very good fellow. In July he and I went for a short walking tour in the Heidelberg neighbourhood.

So far, I had been working as a free lance, without any definite academic end in view. Late in July, as the result of a conference with Sachs and Goebel, I decided to work for the Ph.D. At that time the subject proposed for my dissertation was the relation between leaf and branch. I was advised to take up Physics and Chemistry as the necessary Nebenfächer (or subsidiary subjects).

On returning to Würzburg after the long vacation, I found that

Sachs had changed his mind; he no longer thought the morphological subject suitable. Such questions, he said, were not so much thought of in England as in Germany. I had never taken much to the plan myself, and was relieved when Sachs advised me to transfer my energies to a purely anatomical investigation—the development of articulated laticiferous vessels. This eventually formed the subject of my dissertation.

At that time the development of laticiferous tubes in general was not so well understood as it is now; in particular, the origin of the articulated type by cell-fusion was not finally established. This was the problem which I was to help in solving. Seeds of Compositae and Papaveraceae were at once sown, in order to obtain early stages of development. On Nov. 4th, the first evidence of cell-fusion was secured in the growing-point of *Scorzonera hispanica*.

I attended Sachs's lectures as before, and in addition, those of Kohlrausch and Wislizenus on Physics and Chemistry, my subsidiary subjects. The lectures of Wislizenus impressed me greatly. The Professor was a striking figure; his clear style and brilliant experiments were most effective. The physical lectures, while equally sound, were of a more severe kind, and did not interest me quite so much.

Goebel lectured twice a week on "Entwickelungs Geschichte" (development), a course which appealed to me strongly. In the following February, he reached the subject of Plant-palaeontology; except for a few references in Sachs's text-book, this was practically the first I had ever heard of fossil plants. There were only three lectures on the subject, evidently much influenced by the powerful authority of Renault. I was interested, though it was nearly ten years later before the study of the fossils really attracted me.

During the winter I often read Physics in the evenings with Purdie, afterwards Professor of Chemistry at St Andrews. The Purdies were my chief friends at that time. Otherwise the winter was a rather dreary time of "all work and no play." This was my own fault, as I did not dance.

My research went on with fair success. In February I was able to demonstrate the presence of nuclei in the laticiferous vessels of *Chelidonium*. On May 4th I began writing my dissertation, and finished the first draft on June 1st. It was then submitted to Sachs, who suggested various alterations in the historical part, but quite approved of the original observations. On June 27th he gave his official approval to the finished dissertation. It went to the printers

on July 15th and eventually appeared in the *Arbeiten des botanischen Instituts*, but without illustrations, which Sachs cut out. A translation with some figures was subsequently published in the *Quarterly Journal of Microscopical Science*.

Certain formalities had to be gone through before the examination came off. I had to write my "Vita" and get it translated into Latin by an official scholar. Also a "Gesuch" or formal application for the degree had to be submitted, and "Zeugnisse," or testimonials of courses attended, to be obtained. My old Oxford "testamurs" were also essential, as they enabled me to get off with only three "Semesters" residence. Lastly, on July 18th I had to call on all the Professors of the Faculty, and solemnly invite them to my examination. This formidable ordeal had to be gone through in dress clothes and a tall hat. (It is a pity that an illustration cannot be inserted here!)

The same costume was required for the examination itself, which took place on July 20th, 1881, about the hottest day I have ever experienced. Happily, however, dress clothes are cool.

A German examination in those days was a simple affair compared with our English ones. It was all *viva voce* and lasted two hours. Sachs, as Professor of the chief subject, examined for an hour, Kohlrausch and Wislicenus for half-an-hour each. Sachs's questions concerned the relations between Vascular Cryptogams and Phanerogams, tendrils, twining plants, the movements of *Mimosa* and so on. Kohlrausch examined on Light, the Sun, capillarity, the barometer and steam-pressure; Wislicenus on phosphoric acid, organic acids, sugars, etc. On the whole, a good deal was got through in the time. It was a relief when I was called in at the end, to hear from Sachs that I had passed "summâ cum laude."

Of course the principal thing was to have one's dissertation commended by the Professor of the chief subject. The general *viva voce*, though essential, was of secondary importance.

During this term there had been an important change at the laboratory, for on April 30th Goebel departed, to my great regret, and a few weeks later Adolph Hansen arrived to take his place. The two men were very different and there followed a noticeable alteration in the atmosphere of the department. Goebel had a brilliant career before him, as we all know, and it was of course inevitable that he should soon take up a position of greater independence.

I continued to attend lectures for a few days, and on July 27th heard Sachs's concluding lecture on the History of Botany, which

I find described in my Diary as "magnificent." When I left Würzburg at the end of the "Semester" I was away for nearly a year, during which I was working in England and beginning to take my part in teaching. I returned to the old University about the middle of July, 1882, in order to gain some experience in physiological work. I was accompanied by Walter Gardiner, and the ensuing weeks are memorable for his work, though not for my own.

The problem entrusted to me was a simple, but rather peculiar one. Someone, I think Engelmann, had said that living bacteria could be employed as a test for oxygen, given off by the assimilating organs of plants. I was set to verify this statement. Infusions were made, to obtain the bacteria, and then drops from the cultures were put on the slide with *Spirogyra*, moss-leaves or other green organs. The bacteria swarmed vigorously round the parts where chlorophyll was present. I found that the test succeeded very well, but Sachs never thought much of the method. I remember his asking one morning, somewhat sarcastically, "Beissen die Bakterien an?" ("Are the bacteria biting?"). The effect of different parts of the spectrum on the emission of oxygen, as shown by the bacterial movements, was also tried, but the coloured fluids and glasses used gave no very accurate results.

In the meantime Gardiner was beginning his famous researches on the continuity of protoplasm through the cell-wall. The first suggestion was due to Sachs, who advised Gardiner to investigate the pulvini of motile leaves, and especially the pits on certain of their cells, saying, "It is possible that they are open." Gardiner's immediate results were published shortly afterwards in England; they led on to his more extended investigations, which established the continuity of protoplasm on a broad basis.

On the last day of August we left Würzburg, and that was the end of my botanical experiences in Germany.

The great advantage of working in Germany in those days was that one found oneself in the main stream of botanical progress. Sachs and Goebel, for example, were not only men of first rate ability, they were also the worthy upholders of a great tradition. The revolution in English Botany, carried out at about the time to which these reminiscences belong, was the direct result of German influence; German botanists were undoubtedly the leaders of our Science, at least during the latter half of the nineteenth century. I think, perhaps, we may have been almost too German at that

time; we scarcely gave enough attention to French work, for example. But, on the whole, we drew our inspiration from the right source. At any rate, the result has been to place English Botany on a level with that of any country, and this was certainly not the case in the days before the revolution.

The chief characteristic of German university life, as I saw it, was the dominance of research over mere learning. This, of course, applied to other subjects as much as to Botany. To secure one's degree original work was essential; success depended more on the merit of the research-work than on the acquisition of knowledge. Perhaps this was overdone; some people may have specialised too early, and have failed to acquire a firm grasp of their subject as a whole. Such cases no doubt occurred, but I think they were exceptional. The botanists of Germany, as a rule, were well-informed on all sides of their science. And certainly the consciousness of adding something to knowledge and observing things for oneself for the first time, was a strong stimulus, which tended to keep up the apostolical succession of true men of science.

I feel sure that the English botanists, who in those days worked in the great German laboratories, will recognise, as I do, that they owe any subsequent success in no small degree to the atmosphere of research in which they then lived and moved and had their being.

A CYTOLOGICAL STUDY OF POLLEN DEVELOPMENT IN NOLANA

BY M. G. CAMPIN

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(With Plates I and II)

I. INTRODUCTION, MATERIALS, METHOD

THE present paper describes general cytological conditions during pollen-formation in *Nolana atriplicifolia* and *N. prostrata*, and is a preliminary to further cytological studies on genetic material of *Nolana*.

Both species, natives of Chile, are quite hardy and grow well out of doors in this climate. Buds were fixed at different times during the flowering season in 1 per cent. chrom-acetic solution and acetic-alcohol mixtures, stained by means of iron-alum-haematoxylin and sections cut 8–10 μ in thickness. The cytology of both species was found to be similar; figures are of *N. prostrata*, except where otherwise stated.

2. POLLEN FORMATION

(a) *Prophases of the Heterotype division*

The pollen mother-cell nucleus in rest (fig. 1) shows a single large nucleolus and a wide meshed reticulum of anastomosing threads, slightly thickened at the nodes. It seems clear that the number of nodes or chromatinic aggregations is roughly 12—i.e. the haploid number of chromosomes of the species. When meiotic activity begins these aggregations increase in size and can be counted with greater ease (fig. 2). They are probably of the nature of true "prochromosomes" (11, 19). As the reticulum thickens the nucleolus gives off "buds" of nucleolar material, and at the onset of synizesis this budding becomes more pronounced. Such a process has been described by Miss Allen (2) for *Matthiola incana* and is a rare occurrence among the higher plants. The beginning of synizesis is marked by a contraction of the reticulum at one or more points and a thickening of the reticulum threads (fig. 3). About this time the pollen mother-cells become separate from each other and from the tapetum. Contraction proceeds until a more or less compact mass of chromatinic

material is formed. This is generally adherent to the nucleolus, and lies, in the majority of cases, at one side of the nucleus, in contact with the nucleolar membrane (figs. 4, 5). Synizesis is the term employed in this paper for the height of this contraction, while the term synapsis is reserved for the general period of contraction, from cessation of rest until the end of the segmentation of the spireme. In *Nolana* there is no evidence of the formation of a precipitation membrane surrounding the contracting reticulum: contraction is always very irregular and does not allow for the formation of a delicate membrane such as is described by Gates (10) in *Lactuca* and *Oenothera*. Gates points out that, owing to this membrane, the contracting reticulum "still retains its spherical outline, the evenness of which is an indication of the presence of a limiting membrane." In *Lactuca*, however, the membrane is not always present and its osmotic function not universally established.

(b) *Synizesis and Spireme stages*

Measurements made at the height of synizesis show, as Gates has established for *Lactuca*, that there is a real contraction of the nuclear material, although at this time the nuclear cavity attains its maximum size. The cell is clearly in a very delicate osmotic condition and this probably accounts for the fact that cytomixis is more often seen during synizesis than at any other stage. Synizesis is a phase of some duration; when it is at an end threads begin to appear on the margin of the knot (fig. 6) and soon the spireme emerges as a thin uniform thread (figs. 7, 8). Loops radiating from the nucleolus are unsplit threads with no evidence of parallelism. Free ends are occasionally seen. It is impossible to count the number of loops thrown out, but it is clear in some cases that the sides of a loop curve to lie parallel to each other, and often twist about each other (fig. 10). Although the history of individual loops can only be followed with great difficulty it is evident that certain of them, at any rate, become detached and ultimately form the bivalent chromosomes. Owing to the length and general confusion of the threads it is difficult to affirm that parasynapsis does not take place, but the evidence is certainly strongly in favour of telosynapsis and it is believed that this is the method of pairing.

The spireme, in the stages represented in the figs. 11, 12, is no longer uniform in thickness; parts of it become thickened seemingly at the expense of other parts, and nodules of chromatin are frequently seen. Often certain twisted loops become detached prema

turely (fig. 11). There is no evidence of a second contraction figure, but detachment of the loops, followed by twisting and condensation, ultimately leads to the formation of the 12 bivalent chromosomes. Various stages in progressive condensation are seen in figs. 13-20. That there is ample opportunity for an exchange of chromatinic substance as a basis for genetic crossing-over (18,23) is seen from the figures; in some cases the twisting is of an extremely intimate nature, in others it is of a loose or temporary character. At first the loops are of very different lengths, but condensation leads to the production of chromosomes, which, in diakinesis, are morphologically alike.

(c) *Diakinesis*

Diakinesis is a phase of great precision, the ellipsoidal or rod-shaped chromosomes lie parallel to one another on the periphery of the nuclear cavity. During the time elapsing between diakinesis and the arrangement of the chromosomes on the heterotypic spindle, the chromosomes become still more condensed, their bivalent nature is completely obscured and they assume a more or less spherical shape (fig. 22). At the end of diakinesis the typical form assumed by the heterotype chromosomes is that of a closed ring (fig. 21) but other well-known forms, crosses, Y and V shapes, also occur.

(d) *Heterotype and Homotype divisions*

The heterotypic and homotypic divisions are normal in every respect and need little comment (figs. 22-30). A precocious arrival at the poles of one or more chromosomes during anaphase of both divisions is frequent, but has no apparent significance. The chromosomes never lose their identity during interkinesis, but appear, already split for the homotype division, equally spaced on the periphery of the nuclear cavity and joined by fine anastomosing threads. During the metaphase of the homotype division all evidence of the split is again lost and the chromosomes are completely spherical. The equatorial plates in metaphase may be in the same or in different planes. When the nuclei are reconstituted at the end of the homotype division the chromosomes still persist as separate entities within the nuclear membrane (fig. 31).

The striking manner in which chromosome identity is retained throughout the heterotype and homotype divisions in both species is noteworthy and may, perhaps, be correlated with the rapidity which is characteristic of the process in *Nolana*. Observations show that under favourable conditions both divisions are completed in a

few hours and the fact that the chromosomes pass so rapidly from one phase to the next may provide an explanation of this characteristic feature. The suggestion receives some support from investigations made by the writer on certain slow-flowering species, e.g. *Paeonia*, *Anemone*, etc. where meiosis was of some considerable duration, and was accompanied by loss of chromosome identity during interkinesis.

(e) *Tetrad and Pollen grain formation*

Pollen tetrads are formed by invagination of the cytoplasm. Constrictions of the cytoplasm appear at four places on the periphery and become progressively deeper until they meet, forming four separate microspores (figs. 32-38). This condition is not common in plants but has been found by the writer in several genera of the Solanaceae, and has been described by C. H. Farr(8) in *Nicotiana* and *Magnolia*, by W. K. Farr(9) in *Cobaea scandens* and by Gates(10 c) in *Lactuca*.

3. DEVELOPMENT OF THE TAPETUM

The tapetum, during early archesporial stages is uninucleate (fig. 42). The nucleus contains several large nucleoli which "bud" off nucleolar material; secondary budding also occurs (fig. 43). Mitotic division in the tapetum takes place during very early spireme stages of the pollen mother-cell (fig. 43) and the cell remains bi-nucleate; rarely it becomes tetranucleate. Immediately after mitosis the chromatin aggregates into roundish masses; approximate counts indicate that these have the same number as the somatic number of chromosomes, i.e. 24. Such an aggregation is, however, only temporary, and the chromatin afterwards assumes other arrangements (fig. 45). During heterotype and homotype mitoses of the pollen mother-cell the nuclei of the tapetum become increasingly hyperchromatic in appearance. Finally they decrease in size, and the chromatin coalesces into a tight mass (fig. 47) and finally degenerates (fig. 48). No plasmodium is formed(18, 21 a). The history of the tapetum is not unlike that of *Lactuca*(10 c), but no evidence of a synaptic contraction in the tapetal nucleus of *Nolana* has been found.

4. CYTOMIXIS

The phenomenon of cytomixis is fairly frequent in the pollen mother-cells of *Nolana*, and is considered by the writer to be of normal occurrence. It is most marked in synzesis (fig. 39), but is

also found in spireme stages (fig. 40) and during condensation into bivalents (fig. 41). The transference of nuclear material from one cell to another may be partial, as figured, or complete. Slight cytomixis is very common, and, in the writer's opinion, is not an artefact due to fixative processes (10 c, 22).

The writer wishes to express her warm thanks to Miss E. R. Saunders, at whose suggestion the work was undertaken, both for supplying the material and for her interest in the work, and to Mr F. T. Brooks for his valuable help and advice.

SUMMARY

1. The haploid chromosome number in *Nolana atriplicifolia* and *N. prostrata* is 12 and the cytology of the two species is similar.

2. A process of nucleolar budding in the pollen mother-cell nucleolus at the onset of meiosis is described.

3. There is evidence of the formation of "prochromosomes" in the nucleus of the pollen mother-cell. These appear as aggregations of chromatin on a delicate reticulum.

4. Synzesis, which is a phase of considerable duration, is initiated by a contraction of this reticulum at one or more points and a progressive condensation of the pro-chromosomes. There is no evidence of the formation of a precipitation membrane surrounding the reticulum.

5. The spireme emerging from synzesis is a single thread which throws out a number of loops into the periphery of the nuclear cavity. The sides of each loop come to lie alongside and twist about each other. Loops become detached and condense to form the bivalent chromosomes. It is concluded that the method of conjugation is by telosyndesis.

6. Diakinesis is a phase of great precision, pairs of ellipsoidal chromosomes lying equally spaced on the periphery of the nuclear cavity.

7. Heterotypic and homotypic divisions are quite regular; during interkinesis there is no loss of identity of the chromosomes, and in the tetrad nuclei chromosomal aggregations are still clearly recognisable.

8. Pollen-grain formation by means of furrowing is described.

9. The history of the tapetal cells is outlined.

10. Cytomixis is fairly common and is found in various stages during meiosis. It is considered to be of normal occurrence.

DESCRIPTION OF PLATES

Plates I, II, figs. 1-48a.

All figures, except 13, 16 are of *N. prostrata*. Drawn with a camera lucida under a Zeiss 2 mm. oil immersion lens, t. l. 152, compensating oc. 12 or 6, giving original magnifications of 3000 and 1500 respectively. All figures except figs. 32-48 are drawn with no. 12 oc.

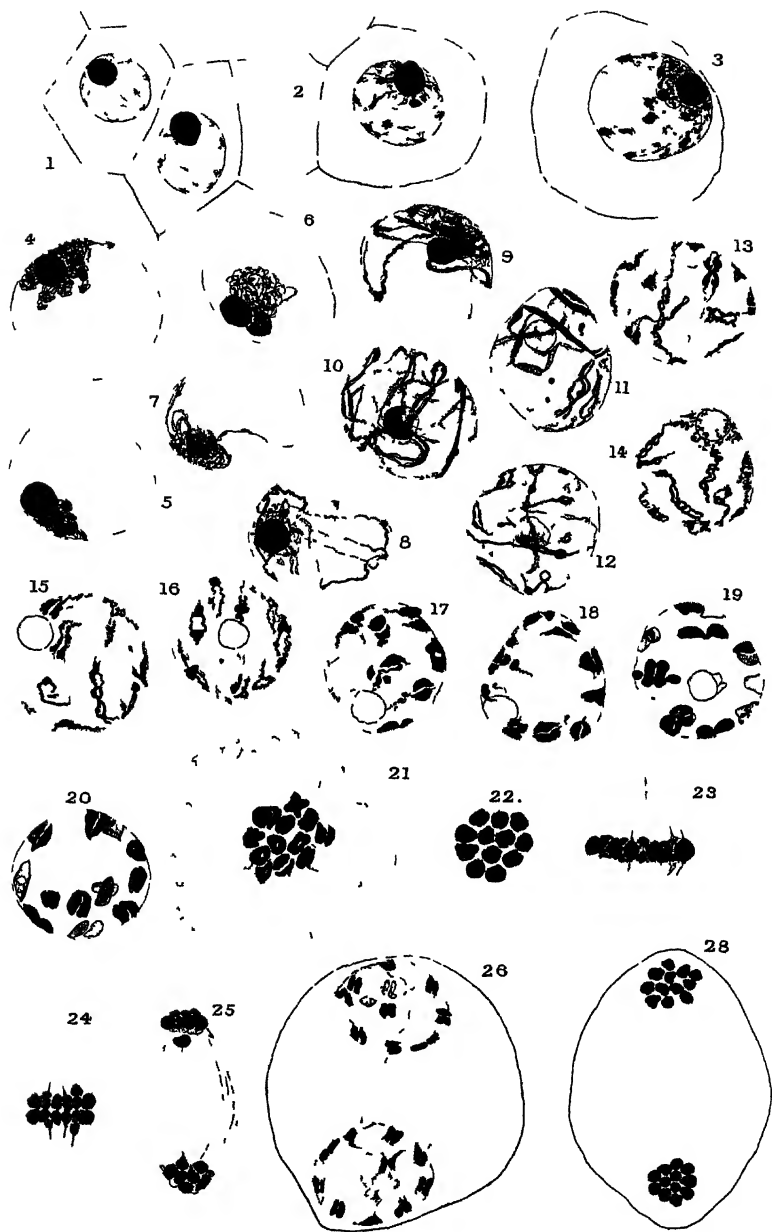
A reduction of $\frac{1}{3}$ is made in reproduction.

PLATE I

- Fig. 1. Pollen mother-cells with nucleus in resting stage.
 Fig. 2. Later stage, showing characteristic aggregations and nucleolar budding.
 Fig. 3. Pollen mother-cell showing beginning of contraction of the reticulum.
 Fig. 4. Reticulum almost completely broken down.
 Fig. 5. Typical synizesis stage.
 Fig. 6. Spireme emerging from synizetic knot.
 Figs. 7, 8, 9. Later stages showing spireme gradually emerging. Free ends visible.
 Fig. 10. Looping of the spireme thread and beginning of twisting.
 Figs. 11, 12. Later spireme stages, showing twisting of thread and characteristic knots.
 Figs. 13-18. Various stages showing segmentation of the thread to form 12 bivalent chromosomes. Figs. 17, 18. Late stages in condensation. Figs. 13, 16. *N. atriplicifolia*.
 Figs. 19, 20. Diakinesis.
 Fig. 21. Nuclear membrane disappeared. 12 bivalent chromosomes.
 Fig. 22. Typical heterotypical metaphase in polar view.
 Fig. 23. Same, side view.
 Fig. 24. Anaphase, early.
 Fig. 25. Anaphase, late.
 Fig. 26. Interkinesis showing 12 split chromosomes within each nuclear cavity.
 Fig. 27. Omitted from Plate.
 Fig. 28. Typical metaphase of the homotype division.

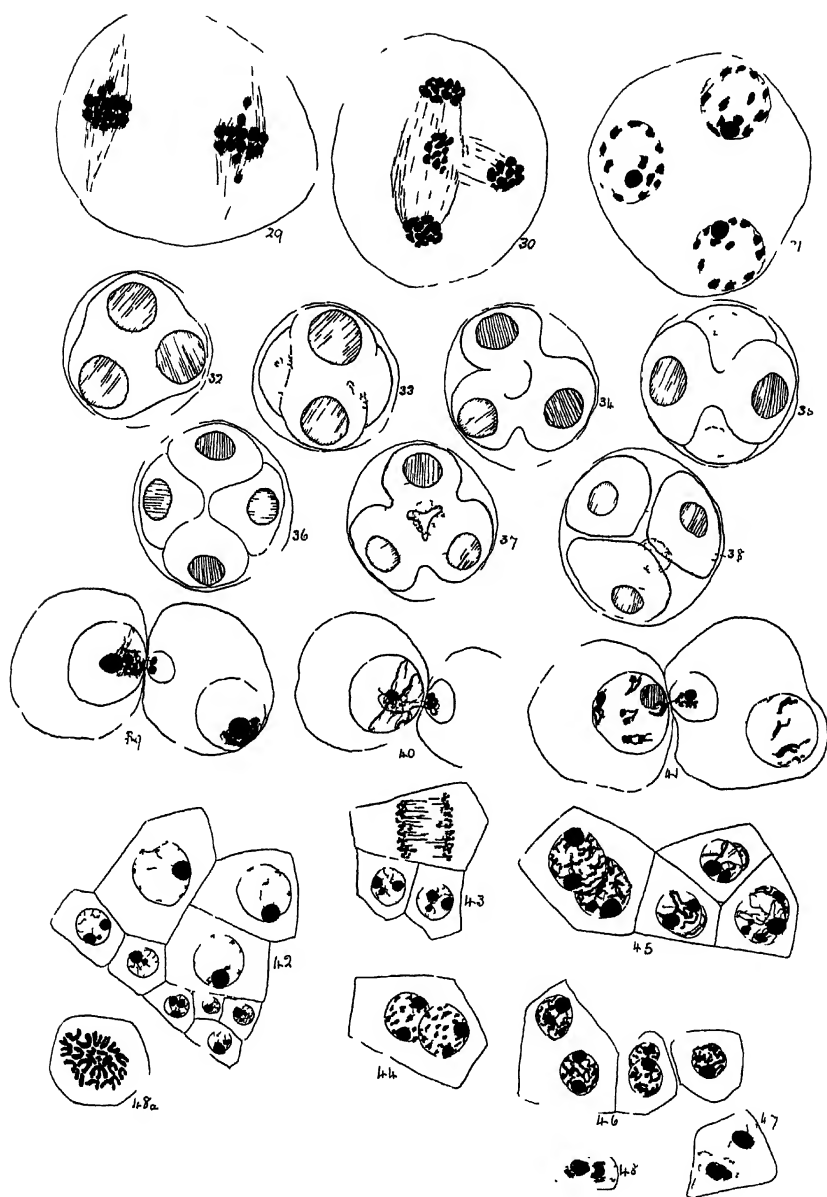
PLATE II

- Fig. 29. Anaphase.
 Fig. 30. End of anaphase of homotype.
 Fig. 31. Nuclei reconstituted, showing no loss of identity of the chromosomes.
 Figs. 32-38. Various stages showing the invagination of cytoplasm to form pollen grains, nuclei shaded.
 Figs. 39-41. Cytomixis. Fig. 39. In synizesis. Fig. 40. In spireme stages. Fig. 41. During the formation of bivalents.
 Figs. 42-48. Stages in the growth and degeneration of the tapetum. Fig. 42. Pollen mother-cells in rest; tapetal cells uninucleate; nucleolar budding in tapetal nuclei. Fig. 43. Mitotic division in one tapetal cell, two still uninucleate. Fig. 44. Tapetal cell binucleate, characteristic appearance of the chromatin during spireme stages in pollen mother-cells. Fig. 45. Appearance of tapetal nuclei during the heterotype and homotype divisions. Fig. 46. Pollen mother-cells in tetrad formation; tapetal nuclei decreasing in size. Fig. 47. Densely aggregated chromatinic material in degenerating tapetal nuclei. Fig. 48. Final stages of degeneration of tapetal nuclei.
 Fig. 48a. Somatic plate from bud tissue.



Hush lth

CAMPIN-POLLEN DEVELOPMENT IN NOLANA

CAMPIN—POLLEN DEVELOPMENT IN *NOLANA*

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ON THE SOLUTES EXUDED BY ROOT PRESSURE FROM VINES

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(With 1 figure in the text)

INTRODUCTION

THE chemical nature of the exudates frequently obtained in considerable quantity from the root system of a plant is a subject of considerable importance in relation to many problems of plant physiology, and it is a matter for surprise that more definite data are not available in the literature. In contributing in the following pages a brief statement of some new data on this subject obtained from considerable quantities of vine sap collected under carefully controlled conditions, the opportunity is taken to indicate the standpoint from which these data have been accumulated and subsequently discussed, and to review very briefly the results of earlier workers.

A very large number of vascular plants of all groups have been shown to manifest exudation pressures from the root system. Wieler (20), in 1893, gave a list of 126 species of 93 genera drawn from Filices, Gymnosperms, Monocotyledons and Dicotyledons, reported by previous workers on what seemed to him unexceptionable grounds; from his own observations he added another 58 species from 47 genera of vascular plants, including *Equisetum*. Extensive observations, such as those of Wieler, provide sound grounds for the generalisation that root systems may manifest this exudation pressure under suitable conditions at any time during the growing season, but that the phenomenon is particularly conspicuous in the spring, when in trees exhibiting this phenomenon strongly, as the vine, the exudation pressure will produce a rapid drip from the most distally placed twigs when cut across, or even drive drops of sap out through hydathodes at the ends of the main veins of the leaf. The mechanism of this exudation is still under discussion (1, 8, 9) and from this point of view the nature of the solutes in the exudate is of importance, but the facts just referred to seem to give this point a wider

significance. This Spring rush of sap from the root system seems closely associated with the vigorous development of the young foliage of the plant. The shoot system at this time is engaged in very rapid synthesis of living substance and consequent meristematic growth, and undoubtedly these meristems derive their food supply, for this metabolic synthesis, from the solutes supplied to them in the sap irrigating them from the termini of the vascular system just below them and driven out to them by this exudation pressure^(10,13).

When the sap is collected from a decapitated stump for analysis, it may contain different solutes to that present in it at the termination of its normal journey below the shoot apex, but the vascular system is clothed by a similar cell system throughout its length and the distance between the end of the vascular element and the meristem is filled by recently vacuolated and relatively impermeable cells so that there is no *a priori* reason for assuming any material difference between the exudate collected and the supply reaching the shoot apex. This being so a close chemical study of this exudate should give valuable indications as to the nature of the food materials with which a shoot apex can carry on active metabolic synthesis and growth. During recent years, in a long series of experiments in this laboratory, not yet published in any detail⁽⁷⁾, the attempt has been made to keep in continued growth the shoot systems of the plant, removed from the root system and supplied under pressure with artificial culture solutions kept as sterile as possible.

Such shoot systems, supplied with sugar and the usual inorganic salts, have remained growing for varying periods, but have never been successful in comparison with the normal growth of the shoot on its own root system. In the light of the data provided below, it will be possible to re-examine the culture solution used in these experiments and to assimilate them more closely to the solutions occurring in nature. Furthermore, whilst various investigators are assuming the presence of growth promoting or growth inhibiting substances, hormones or chalones, in the interpretation of very varied experimental results with plants, it should be possible by experiments along these lines to supply the critical experimental evidence, at present entirely lacking, for the existence or absence of these substances, together with a gradual analysis of their chemical and physical nature, by culture experiments with isolated shoot systems equivalent to the experiments of animal physiologists in feeding synthetic experimental rations to animals.

It is thus of great interest that the data to be presently recorded

make it probable that the organic nitrogenous substances present in the sap of the vine are so minute in quantity that their rôle in relation to growth must be confined either to catalytic action as enzymes, or to growth regulation as a hormone, or as some form of accessory food factor or vitamin.

This result, if established for the present only for the vine, is of very considerable importance, and is in line with some preliminary observations in this laboratory(11) in which a wider range of plants were under observation. Wiegand(19) has attempted to classify the trees showing exudation pressures into two groups; in one group, of which various species of *Acer* are typical examples, bleeding commences very early in the season whilst frost is on the ground, in the other, of which *Betula* and *Vitis* are characteristic members, exudation begins later and seems less influenced by air temperature. Wiegand regarded the flow in *Vitis* and *Betula* as more regular after its commencement, rising gradually to a maximum and then falling until its complete cessation; in *Acer*, on the contrary, the flow was very irregular from day to day, and profoundly affected by air temperature, being especially vigorous in the day when the night and day temperatures are below and above 0° C. respectively.

Wiegand describes the sap of the Vine as almost pure water, containing no sugar, statements clearly incorrect, but he does not attempt to support his classification on this ground, on account of the large percentage of sugar in birch sap. The main grounds for the distinction into two groups lie in the great sensitiveness of the *Acer* type to air temperatures and consequent irregularities in sap flow throughout the period of exudation. This points to an important rôle being played in *Acer* by the living tissues of the shoot, and this conclusion is supported by the downward flow of sap in the Sugar Maple. Schroeder(17) emphasised this latter difference between sugar maple and birch in his early work, and the phenomenon is fully placed on record in the long series of experimental tappings with specially designed instruments made by Jones, Edson and Morse(3).

This would suggest that when sap moves into the shoot with the earliest activities of the root in *Acer*, the result is a vigorous contribution to the solutes in the sap, and thus to the development of exudation pressures, by the living cells of the shoot. Under these circumstances Schroeder's observation that more protein is present in the sap of *Acer* is of great interest and a full chemical comparison of the solutes of a tree of this type would be very valuable; the extensive data collected by Jones, Edson and Morse for the Sugar Maple

refer only to sugar content. Possibly, the necessary data can be extracted from technical publications, but they have eluded the writers.

From the same standpoint it might be possible to explain Miyoshi's observations(4) on the bleeding of *Cornus macrophylla*, apparently the most vigorous example of exudation pressure in the Far East. Here again the pressures vary remarkably during the season, a constant feature in Miyoshi's observation being a very rapid fall of pressure following closely upon the maximum pressure recorded by manometers attached to lateral branches of the tree. We suggest that the first development of a high exudation pressure in the vascular system, produced by root action as the water must enter from the soil, is followed by a sudden fall of pressure due to the liquid in the vascular system finding access, owing to the pressure generated, to the intercellular system in the cortical regions of the shoot. This causes a leakage of sap from the vascular system, which, coupled with the osmotic action of the parenchyma, now that the sap finds access to it, may even produce a negative pressure; the subsequent daily fluctuations suggest that the living cells of the shoot and thus the air temperatures, may then play an important rôle in the exudation in *Cornus*, as in *Acer*.

For these reasons, the present data supplied for *Vitis* may prove on examination to vary widely from results obtained on the examination of *Acer* or *Cornus*, but will probably be found in general accord with the full data for *Betula* when available. For *Cornus macrophylla*, Miyoshi has supplied the following data: dry substance, 0.425 per cent.; sugar, etc. 0.1714 per cent.; inorganic substances, 0.1382 per cent.; substances with amide groups, 0.0246 per cent.; remaining organic substances, mainly organic acids, 0.0908 per cent. Many of the earlier data recorded for *Vine*, *Birch*, *Maple*, etc., are briefly summarised in an earlier paper (Wormall(21)).

EXPERIMENTAL METHODS

With a view to full chemical analysis, as large a quantity of liquid as possible was required with every possible precaution taken to avoid contamination, together with avoidance of fermentation by micro-organisms, which under natural conditions promptly follows as the sap exudes from a cut stump (Ogilvie(6)). It was decided, therefore, to collect as large quantities of sap as possible and examine it in bulk, making no effort for the present to distinguish the composition at different stages. Such work can be so much better directed when full chemical examination provides a clue to the nature of the

great majority of substances present. A large number of well-established vines were available, grown in greenhouses attached to one of the University hostels, Oxley Hall. The root systems of the vines were in some cases in the ground outside the house; in another house younger vines employed were planted within the house, but the houses were not heated during the experiment. Twelve vines were taken in all, the cutting of the shoot and its connection to a collecting system under antiseptic conditions proved a tedious business, in which the senior author was assisted by Professor H. S. Raper. The first vines were cut upon April 5th, 1923, work being continued when convenient during the next fortnight, until all vines were prepared. Some of the vines were still bleeding slowly until June 17th, when the process of collection was finally stopped.

Previous attempts to collect a sterile juice⁽¹¹⁾ under toluol had provided experience in the light of which the present more elaborate precautions were taken, the Vine limb now being removed practically with the precautions adopted from surgical experience and, in the main, suggested by Professor H. S. Raper. Each vine-stem was sawn across about 1-2 feet from the ground, the saw being previously sterilised in the flame, after cleaning with an alcoholic solution of K_2HgI_4 . Before sawing, the bark, in a region chosen for its relative freedom from surface irregularities, was carefully trimmed off, great care being taken not to injure the phloem, and the trimmed surface wiped thoroughly with the same alcoholic solution of K_2HgI_4 . The cut surface was then trimmed rapidly with a sterilised broad-bladed knife and stem and cut surface again wiped with the alcohol solution of K_2HgI_4 .

A metal cap, a turned iron cylinder about 2 inches in length, with a side tube of composition tubing, was then attached to the stump by means of a rubber collar, a portion of either a motor cycle or motor car inner tube, depending upon the thickness of the stem. The metal was previously sterilised in boiling water, the rubber collar immersed in the alcoholic K_2HgI_4 just before use. A pad of cotton-wool soaked in toluol was placed in the end of the metal cap before its insertion. The rubber collar and cap were firmly bound on to the stem with adhesive, antiseptic surgical tape, over which thick copper wires were fastened very firmly by pliers. If the roughness of the stem surface necessitated it, this surgical tape was bound down upon pieces of plasticine inserted into the grooves on the stem. When pressure was then brought upon this by tightening the wire, it proved possible in every case to obtain finally a water-tight join.

The composition tube was led immediately to a glass Winchester, in which a rubber cork with two tubes was already inserted, one of these tubes being fitted with a short length of tubing, and both closed with cotton wool. All these Winchesters were previously sterilised by prolonged heating in a steam steriliser in the University, and now, before the composition tube was joined to the rubber tubing, after

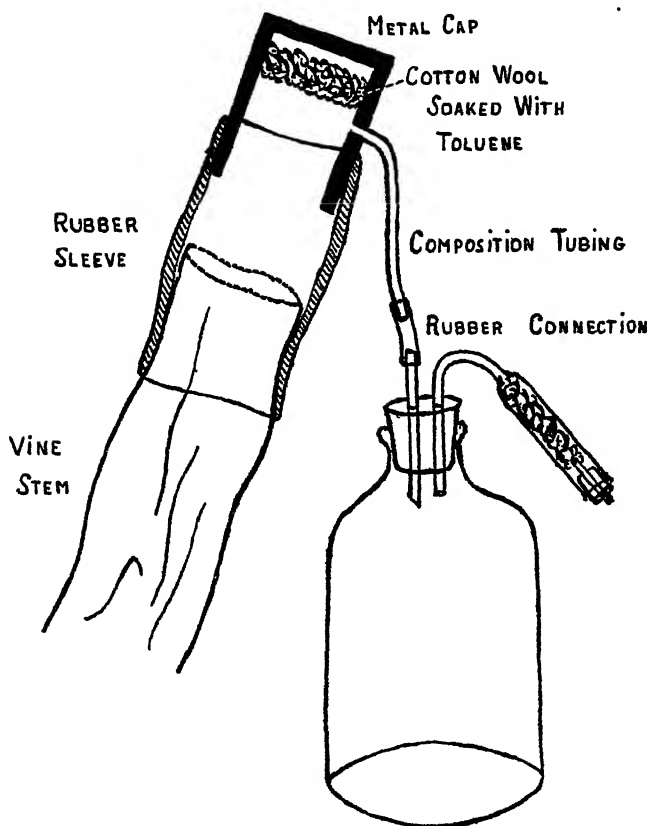


Fig. 1. Apparatus in position

removal of the cotton wool, some 100 c.c. of toluol were run into the collecting vessel. As the sap was collected, the displaced air passed out of the Winchester through the second tube, which was bent downwards so that the cotton wool in it should not become wet through dripping from the greenhouse roof (Text-fig. 1). As one Winchester filled it was replaced by another with the same pre-

cautions, the filled Winchesters being removed as soon as possible from the glasshouse and stored in a dark cellar until required. Some of the samples were water clear, the contents of other bottles opalescent. Observation showed this opalescence to develop rapidly in the clear solution on exposure to light and to have no connection with bacterial contamination, subsequently therefore so far as possible the liquid was collected either in dark glass Winchesters, or in bottles shaded by brown paper or sacking.

The twelve vines yielded about 110 litres of sap; 90,300 c.c. of this were used for analysis, the dilute sap being concentrated by distillation at a temperature of 50–55° C. under a pressure of 20–30 mm. Whenever the distillation was stopped, toluol was added to the concentrated juice, and as a precautionary measure, the air bubbled through the liquid to assist distillation, was filtered through cotton wool. As the liquid concentrated, a brownish-grey deposit separated on the side of the three flasks used in the distillation. When the 90,300 c.c. had been concentrated to 1205 c.c., the distillations were stopped, the solutions filtered and the precipitate washed with a small quantity of distilled water.

The concentrated liquid was stored in an ice-chest, toluol being added during the whole period of examination.

From time to time, the solution was kindly examined by Dr H. V. Phelon of the Department of Bacteriology, and no vegetating organisms were ever found present in it. From the nature of the case, bacterial spores can never be completely excluded in the collection of such an exudate as this, but it can be said with confidence that at no time during the collection or subsequent examination of this liquid was opportunity provided for the development of the organisms and that the chemical results obtained are free from misrepresentation due to secondary changes produced by micro-organisms.

EXPERIMENTAL RESULTS

Both distillate and concentrated solution and the precipitates obtained during concentration were fully examined, the chemical procedure having been set forth in detail by one of us elsewhere (21).

It is not proposed to repeat these details of method here, but briefly to summarise the results obtained from an examination which was made as critical and detailed as the material permitted.

The sap is a dilute solution containing 1.56 gm. of solid matter per litre of which approximately one-third is inorganic and the remainder organic. The organic constituents are chiefly sugars and

organic acids, the sugars present being glucose, fructose and a very small amount of cane sugar; there is also probably a trace of galactose.

The organic acids present are oxalic, tartaric, malic and succinic, the total quantity of these acids exceeding that of the sugars present. A small amount of an acid with M.P. 161-162° C. was isolated, but not identified. The mineral salts present are the chlorides, sulphates, nitrites, nitrates, silicates and phosphates of sodium, potassium, calcium, iron, magnesium, and, to a lesser extent, of manganese and aluminium. The iron present is readily diffusible, and passed through a collodion sac, or a parchment dialyser.

Only the merest trace of organic nitrogen could be detected, practically the whole of the nitrogen being present as nitrates and nitrites; this trace of organic nitrogen seems barely adequate to account for the enzyme activity of the juice.

No lipins could be detected but a mere trace of a substance with the solubilities and characteristics of fat or fatty acid was isolated.

Of enzymes, diastase, peroxidase and a trace of catalase are present, maltase, invertase, lipase, protease, glycerophosphatase and rennin were absent. In the following table, as precise a statement as possible is made as to the quantities of these different substances in the Vine exudate; in the first column the total amount of substance in the 90,300 c.c. of liquid is given, in the second column is given the calculated concentration of the substance in the original sap.

TABLE I

Substance	Total amount	Amount in grams. per litre of natural sap
Ash	50.3	0.56
Total solid	140.7	1.56
P ₂ O ₅	2.92	0.032
Reducing sugars	29.64	0.33
Glucose	26.03 ¹	0.29
Fructose	3.61 ¹	0.04
Cane sugar	0.73	0.008
Inorganic N	2.11	0.023
Organic acids (oxalic, tartaric, malic, and succinic) approx.	50	0.56

¹ Ratio of glucose/fructose from polarimetric readings after the removal of organic acids.

DISCUSSION

The general standpoint from which these results are regarded has been indicated in the introductory section, and in the light of these

preliminary remarks, it is now proposed to pass in review the main results summarised above.

Concentration. It is clear that the exudate is much less concentrated than that frequently described, even from the same plant, though it is far from deserving the expression "pure water."

Quantitative tests confirmed the statement of Priestley and Armstead(11) that in the earlier part of the bleeding period the juice is considerably more concentrated in sugars, the organic matter and especially the sugars falling off in amount as the season advances, while the ash remains approximately constant in amount.

This result is obviously in accord with the view that the salts are entering the sap from the soil originally by a steady process of diffusion, the organic matter on the other hand, supplied from the organic reserves of the plant, is falling off in amount as the supplies are exhausted. That this decline in organic matter accompanies the gradual fall in rate of flow is at least in accordance with the view that the osmotic properties of these solutes, unable to escape from the central cylinder through the protoplasts of the 'endodermal cylinder, or through the fat impregnated network of the Casparian Strip(12), are associated with the production of the flow.

Moreau and Vinet(5) have given some interesting data of the proportions of ash, sugars, and other organic matter from bleeding vines, and their figures, some of which are given in the following table, suggest that very considerable variations occur during the period of exudation, and that these are connected with external factors, especially temperature.

These interesting data, given in full as they are not readily accessible to botanists, show the same relative constancy in ash content, the variations showing a rough inverse proportionality to rate of flow, rapid flow being associated with relatively low ash content, provide further support for the view that, in the main, the ash moves into the sap at a comparatively uniform rate by diffusion.

The fluctuations in the organic matter are, however, very much more striking. Moreau and Vinet conclude that the organic matter found present strikes the balance between the amounts mobilised in the root and utilised in the shoot region under the conditions of experiment. Under frost conditions mobilisation continues, but utilisation is reduced in amount and therefore the amount of organic matter formed rises.

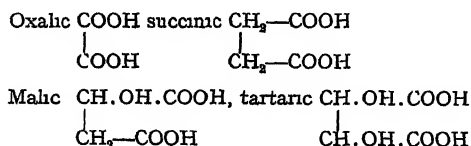
Mobilisation continues because the deep-seated root system is little affected by the cold spell.

TABLE II (from data of Moreau and Vinet)

Condition and date		Temp. (mean) Max. Min.		Mean volume collected in 24 hours per vine	Concentration given in grams per litre			
					Dry matter at 100° C.	Ash	Reducing substance calc. as glucose	Other organic matter
1921. <i>Several Vines (buds opening during frost)</i>								
Before frost—								
April	9-14	19.0	2.3	—	1.11	0.34	0.25	0.52
During frost—								
April	15-22	12.5	-1.1	—	5.59	0.52	3.12	1.95
After frost—								
April	23-30	19.1	4.6	—	1.36	0.31	0.24	0.81
1922. <i>One Vine (before buds open)</i>								
March	20	6.0	4.0	144	1.90	0.56	0.38	0.96
"	20-21	6.5	1.0	115	2.24	0.54	0.73	0.97
"	22-23	0	-2.0	19	12.30	0.70	10.80	0.80
"	23-24	5.0	-3.0	16	13.10	0.80	11.02	1.28
"	24-25	8.5	1.0	22	6.10	0.65	4.00	1.45
"	28	9.0	5.0	63	1.7	0.70	0.22	0.78
"	30-31	11.0	1.0	71	3.88	0.40	3.08	0.40
1923. <i>Three Vines (before buds open)</i>								
March	7-10	13.0	6.0	38	1.13	0.38	0.06	0.69
"	10-12	10.0	0.0	31	5.16	0.36	3.71	1.09
(frost)								
"	16-17	12.0	5.0	35	0.84	0.28	0.03	—

Proportion of Sugars in Organic Matter. Moreau and Vinet regard the sugar as arising directly from starch. Reasons have been given in this Journal recently (14) why this view is regarded as unfounded and why the sugar content is regarded as released from the differentiating protoplasts which surround the xylem system on all sides and which add continually to its mass during the growing season. It is suggested, therefore, that the high sugar content during and immediately after a period of low night temperatures is the result of continued growth during the period associated with slower flow of water, so that the liquid becomes more concentrated, and with lessened respiratory destruction of the sugar, so that the proportion of sugar rises in comparison with organic acids. On the other hand, a study of the table shows that in every case a comparatively high minimum temperature is associated with a high proportion of organic acids, relatively to the sugar, and this seems to indicate that the respiratory destruction of the sugars is associated with a moderate rise of temperature.

The Organic Acids. The organic acids described as present in the previous section are of great interest. Apart from the small quantity of an unidentified acid present, they are all di-basic, thus



We know practically nothing of the fate of sugar in the metabolism of the normal plant, and a study of its fermentation by yeast has somewhat concentrated attention upon the three carbon chain method of breakdown in which lactic acid is to be expected, but tests for this acid were completely negative.

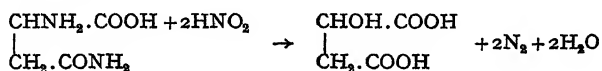
Raistrick and Clark⁽¹⁶⁾ have provided us with one clear example of a fermentation by *Aspergillus niger* in which the di-basic oxalic acid is the main product. In this case these other di-basic acids could be readily utilised (tartaric acid with some difficulty) if supplied in the culture medium and their disappearance was accompanied by the appearance of oxalates. It is therefore possible to regard these acids provisionally, as arising from the oxidation of the sugar, although alternative methods of origin are not excluded, another being referred to on p. 35.

However they arise, their presence certainly clears up a point referred to in a former paper⁽¹¹⁾. Miss L. M. Woffenden had found that the hydathodes in the leaf teeth of *Fuchsia* passed, during development, through three stages. In the young leaf they were unopened; they then passed through a period in which free guttation took place to become blocked later by an amorphous deposit which, from micro-chemical examination certainly seemed to be calcium oxalate. Furthermore, in the neighbourhood of the leaf hydathodes of a similar type in many other leaves, crystalline deposits of calcium oxalate are abundant. These observations would suggest that the presence of oxalates was of frequent occurrence in the liquid driven into the shoot by the root and forced out through the hydathodes under pressure.

Nitrogen. As pointed out in the introduction, the most striking feature of the result is the very small trace of organic nitrogen. No importance would seem to attach to the exact ratio, as determined, of NO_2/NOO_3 in the sap. Obviously this ratio would be liable to fluctuation if the liquid were exposed to light and the only con-

clusion of importance in reference to inorganic nitrogen is that, even when precautions are taken to collect the liquid in the dark, appreciable quantities of nitrite are present and must be assumed to be present in the sap rising normally in the stem, arising possibly from enzyme action (Irving and Hankinson(2)), possibly from reaction of nitrates with sugar under suitable conditions in which the sugars undergo some oxidation.

A certain type of nitrogen organic compound may be absent from the sap, owing to the presence of the nitrites in a juice which, when collected is neutral or slightly acid ($pH\ 7-pH\ 6$) in reaction and which, during its progress up from the root has very probably been in its early stages acid in reaction. Asparagin in an acid solution would then react



giving rise to malic acid. This possibility must be borne in mind as well as the suggestion that the malic acid arises from the oxidation of the sugar.

Enzymes. These results are of interest, whether negative or positive. The presence of diastase, which has also been identified in the exudate of the Sycamore(15), is of very great interest in view of other work in progress in this laboratory. Mr Swarbrick finds that during the season in which root pressure can usually be demonstrated in certain trees, the result of cutting a branch from any woody plant studied is that a block gradually forms in the vessels below the cut surface, and that in the region just below this, the starch then disappears from xylem parenchyma and medullary rays. In the rhododendron, where this process was followed very closely one summer, this starch had completely disappeared within fifteen days of making the wound.

Further down below the block this starch still remains. It is hoped to publish fuller details of this work shortly, and in this connection the significance of the presence of diastase in the exudate of both Vine and Sycamore hardly needs stressing. Votchal(18) has also reported diastase in the sap gathered from trees.

A feature of the peroxidase present is its extraordinary stability. This sap was obtained in June 1923, and throughout 1924 the peroxidase, both in concentrated and normal sap, retained its activity.

Maltase would not be expected to be present in such dilute sap

in view of the difficulty that is always experienced in separating this enzyme from its solid substratum.

Invertase, on the other hand, is very soluble, and its absence is perhaps surprising; it has been found to be present in the sap of the sycamore. As a result of Mr Swarbrick's work, referred to above, there would appear to be an *a priori* case for the presence of a pectin-digesting enzyme, possibly a pectinase, and in the coming Spring a search will be made for this enzyme.

SUMMARY

1. The chemical determination, qualitative and quantitative, of the solutes present in the sap driven up from the roots by exudation pressure is of interest, not only in elucidating the mechanism at work in developing this exudation pressure, but also as throwing light on the substances essential in promoting and maintaining growth in the shoot of the plant.

2. The data at present available are scrappy, and whilst they suggest that two types of tree may be distinguished in regard to the distribution and nature of the exudation pressures manifested, they do not enable a decision to be reached on this point.

3. The suggested distinction would be between (1) trees like the Vine in which the pressure is always upwards from the root, and in which the flow of sap usually rises to one maximum, and then in the course of weeks subsides, and (2) trees like the Sugar Maple, in which the flow is downwards as well as upwards, in which flow and pressure fluctuate much more greatly and seem to be connected closely with the air temperature.

4. It is suggested that in the case of the Acer type, the result of the development of an exudation pressure by the root in the Spring may be the irrigation of the cortex of the shoot by the sap, with as a result considerable contribution to flow and pressure subsequently as a result of the activity of the parenchymatous region of the shoot, the sensitiveness of flow and pressure to air temperature being a natural consequence.

5. Under these circumstances the chemical nature of the sap exuding from Acer may well differ from the Vine and attention is drawn to Schroeder's report of more protein in the sap of Acer.

6. The methods are described by which large quantities of vine sap were collected under sterile conditions from twelve vines.

7. On pp. 43 and 44 are summarised the chemical results of the

examination of this sap. They are not repeated, as they hardly admit of further condensation.

8. The concentration of the sap in organic and inorganic matter is discussed and data quoted from Moreau and Vinet which show that the concentrations of organic matter are very variable, and seem to be affected, both in quantity and in proportions of chief constituents, by external factors, especially temperature.

9. The presence of several dibasic acids, practically to the exclusion of any other type, is emphasised and discussed.

10. The absence of all but trace of organic nitrogen is a striking feature of the results. Amino-acid amides, such as asparagin, could not be expected to be present in a juice containing nitrite, which is probably acid in reaction in the root.

11. The presence of the enzymes diastase and peroxidase is briefly discussed.

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MORPHOLOGICAL QUESTIONS FROM A RUSSIAN POINT OF VIEW

By D. H. SCOTT, F.R.S.

I HAVE received from Dr V. Lashevsky, lecturer on Botany in the University of Woronesh (formerly Dorpat), Russia, some notes on morphological questions, under the general title: "Notes on the comparative morphology of microphyllous Cormophyta." They are of a theoretical nature, and not based on any original observations. As Dr Lashevsky tells me, he intended his notes to be an introduction to his own original work; they were written "during the firing of the civil war," and, even when that ceased, there was no possibility of commencing original work. It has not been found practicable to publish Dr Lashevsky's essays in full, but, under the circumstances, the Editor kindly authorises me to write an article for the *New Phytologist*, giving the substance of our Russian colleague's remarks, with such comments as suggest themselves.

I. THE ULODENDROID SCAR

The first note is on the Ulodendroid scar, a subject which has been much in dispute among palaeobotanists. The large round scars are ranged alternately in two vertical rows on opposite sides of the stem in various Lepidendroid plants of Carboniferous age. For a long time, it was commonly held that large cones were attached to the scars, but the cones are now known to have been otherwise borne. Of late

years the view has gained ground that caducous lateral branches have left their mark in these scars. While Prof. Watson holds that the base of the branch was attached to the whole area of the scar, Prof. Renier has maintained that the branch was attached only to the umbilicus, the more or less central print within the scar. The marks on the peripheral area of the scar no doubt indicate vascular traces, and their characters are more favourable to Prof. Watson's view¹.

Dr Lashevsky has his own interpretation, and suggests that root-like organs (rhizophores) were attached to the Ulodendroid scars. The organs in question would thus be equivalent to the main axes of *Stigmaria*—the so-called "roots" of the Lepidodendreae.

Mr Carruthers, as long ago as 1870, had expressed the opinion that the appendages attached to the scars must have been adventitious roots. His conclusion, however, was based on a misinterpretation of a specimen which was not even a *Ulodendron*, but an "*Halonina*"².

In the case of true Ulodendroid stems, the characters of the scars show that where the appendage was obliquely inserted its inclination

Dr Shattock, in 1887, considered the possibility that roots or rhizophores might have been borne on the scars. Sir W. T. Thiselton-Dyer, in a note appended to Dr Shattock's paper, called attention to the rhizophores of *Selaginella*, and added that he saw nothing improbable in the suggestion that the scars of *Ulodendron* may have belonged to rhizophores; "the view is, at any rate, one which is worth consideration"³.

Dr Lashevsky's theory is essentially the same as that suggested in the passage quoted; he supports it by arguments of his own. His first contention is, that the Ulodendroid scar corresponds in its features to the transverse section of a Stigmarian root or rhizophore. He points out that if we suppose that the Stigmarian roots had been

¹ For this controversy see. D. M. S. Watson, "On the Ulodendroid Scar," *Manchester Memoirs*, 52, 1908; A. Renier, "L'Origine Raméale des Cicatrices Ulodendroides," *Ann. de la Soc. Géologique de Belgique*, T. 11, 1910; D. M. S. Watson, "On the Structure and Origin of the Ulodendroid Scar," *Ann. of Botany*, 28, 1914.

² Carruthers, "On the nature of the scars on the stems of *Ulodendron*," etc., *Monthly Microscopical Journal*, 3, 1870, see p. 150 (in many respects a very valuable paper). For the correction of the error, see Kidston, "On the relationship of *Ulodendron*," etc., *Ann. and Mag. of Nat. Hist.* 16, p. 240, 1885.

³ S. G. Shattock, "On the Scars occurring on the stem of *Dammara robusta*," with supplementary note by W. T. Thiselton-Dyer, *Linnean Society's Journal, Botany*, 24, 1887.

shed on the base of the stem, there would be scars like those of *Ulodendron*—the wood in the centre, the broad zone of cortex around, the well-preserved trabeculae of the middle cortex, and the woody part of the free rootlets; these would give the characteristic features of the Ulodendroid scar—radiating ridges, spirally arranged prominent points, etc.

It may be mentioned that although well-preserved trabeculae may occur in the rootlets, they have not been observed in the main Stigmarian roots, which are alone in question here. Further, as has often been shown, the radiating ridges and prominent points of the Ulodendroid scar are identical, both alike representing the prints of vascular strands, but intersected at different angles, owing to the oblique insertion of the appendage. This, however, is no serious objection to Dr Lashevsky's interpretation; the radiating or punctiform prints might quite possibly represent the bundles of rootlets, instead of leaf-traces. A more serious difficulty arises as regards the umbilicus, which Dr Lashevsky evidently interprets as representing the wood of the root. The dimensions, however, do not seem to agree very well. The umbilicus, as Prof. Watson has shown, is never more than a quarter of the diameter of the scar, and, as a rule, only about a fifth. In large Stigmarian roots, of a size comparable to the Ulodendroid scars, I have found the wood well developed, measuring perhaps half the entire diameter of the organ.

The umbilicus of the scar is often in an eccentric position. Dr Lashevsky accounts for this by displacement, owing to decay of the delicate tissue of the middle cortex. The position of the umbilicus appears, however, to be too regular and constant for this explanation. When not central it lies below the centre and in the median plane, a position explained by the obconical form of the scar, combined with the oblique insertion of the appendage in such cases¹.

An obvious objection to the Stigmarian theory of the Ulodendroid scars is the way in which *Stigmaria* is known to have been attached to the stem. Where a Lepidodendroid stem is found in connection with its Stigmarian roots, the latter are always four in number, running out at equal angles from the base of the tree. This seems to accord ill with the constant arrangement of the Ulodendroid scars in two series only, but Dr Lashevsky ingeniously meets the difficulty by adopting the view that there are really only two roots, in the typical case, which branch by dichotomy at the very base. He is thus enabled to interpret the Stigmariae actually found in

¹ See Watson, p. 493, 1914.

position as the lowest pair of roots, forking at once so as to simulate four. He supports this suggestion further by citing a *Ulodendron* described by Prof. P. Bertrand, in which each scar has two umbilici—evidence of an early dichotomy.

But the fact remains that the Stigmarian roots actually found in connection with a stem would give rise, if detached, to four scars at the same level, an arrangement quite different from that of the biseriate *Ulodendroids*.

Analogies drawn by the author with the adventitious roots of *Calamites* are evidently too remote to affect the immediate question.

On the other hand, the comparison with the rhizophores of *Selaginella*, already emphasised by Thiselton-Dyer, is undoubtedly to the point, for it shows that there is no inherent improbability in a Lycopod bearing root-like aerial organs on its stem.

Dr Lashevsky's hypothesis is not an impossible interpretation of the *Ulodendroid* scars. But there is very little evidence to support it, and it is difficult to see what advantage the theory of rhizophore-scars has over the now current view, that the shedding of ordinary caducous branches gave rise to the *Ulodendroid* condition.

II. THE LIGULE OF LYCOPODIALES

The subject of Dr Lashevsky's second note is the ligule of fossil Lycopodiales. The ligule, as is well known, is constantly present on the leaves and sporophylls of the recent heterosporous Lycopods *Selaginella* and *Isoetes*; it was of general occurrence, in much the same form, among the Palaeozoic Lycopods, as in *Lepidodendron* and various other genera.

Dr Lashevsky points out the difficulties encountered in determining the function of the ligule, in the recent *Ligulatae*; it has been interpreted in different cases, as a mucilage-secreting, a water-secreting and a water-absorbing organ. The author explains this inconstancy of function by the ligule being a vestigial organ. This must have been the case, on his view, ever since early Carboniferous, if not Devonian times.

Dr Lashevsky's interpretation of the ligule and of the Lycopod sporophyll, as a whole, is based on the analogy of Palaeozoic Calamarian fructifications. In *Calamostachys* the axis of the cone bears whorls of sporangiophores, separated from one another by alternate whorls of bracts. Here the successive verticils are equidistant. In *Palaeostachya*, on the other hand, the whorls of sporangiophores are inserted immediately above the bracts and in their axils. The author

considers that this was an improvement on the *Calamostachys* plan, as it gave better protection to the sporangia. This may be so, but, for all we know, the one arrangement worked as well as the other.

However that may be, Dr Hickling has inferred, from the course of the vascular strands supplying the sporangiophores, that the *Palaeostachya* type was derived from that of *Calamostachys*. This provides the text for Dr Lashevsky's discourse. "The study of fossil Equisetalean fructifications suggests that the sporangiophore moved into the bract-axil for its protection, during the phylogenetic development of these plants."

How, then, is this conclusion to be applied to the interpretation of the Lycopod sporophyll? Dr Lashevsky says: "On the ground of its morphological and anatomical characters the sporophyll (in *Lepidostrobus*) is properly a bract; it is often called by this name. But this bract has a superstructure on its upper surface; a narrow band of tissue, with which both sporangium and bract are connected, and a ligule."

This superstructure, including the ligule, is regarded as representing the original sporangiophore, now closely fused with the underlying bract. "The ligule, with the tissue to which the sporangium is attached are its exterior traces, and the branch-bundle (running up to the ligule) with the parichnos are its internal traces. The whole *Lepidostrobus* fructification is a further stage of evolution of the type *Palaeostachya*." And further on: "The explanation of the significance of the ligule as a rudiment of a terminal part of the sporangiophore, which was at first lowered on to the bract-axil and then united with it, entirely accords with the process of evolution in the fructification demonstrated by Dr Hickling for the Equisetales." The author regards the early development of the ligule in recent *Ligulatae* "simultaneously with the sporangium, long before the development of the leaf, as in agreement with the view that the ligule is a rudiment of the sporangiophore."

There are two objections to Dr Lashevsky's theory. In the first place, it is based entirely on a comparison between certain Equisetales and the ligulate Lycopods. There is not the slightest reason to suppose that the two groups are related and the argument amounts at most to an analogy, if even to as much as that.

The sporangiophores of *Palaeostachya* are no doubt inserted immediately above the bracts, apparently in their axils. They may have shifted into this position from a more independent insertion on the internode above, but this is still an open question. There is no

evidence of any fusion between sporangiophore and bract, nor of any reduction in the former. Of course, such changes might occur—if they did there is no reason to suppose that anything resembling a ligule would result. On the whole, I cannot see that the Calamarian analogy affords any solid support to Dr Lashevsky's theory of the Lycopod cone.

The other serious objection is that his theory of the nature of the ligule applies to the vegetative leaf just as much as to the sporophyll. In fact, in speaking of the fossil Lycopods, he bases his account on the leaf, because he had better data for this than for the sporophyll. We have to assume then, that every leaf of the Ligulatae, from the cotyledon onwards, bears a reduced sporangiophore! This seems a tremendous assumption, though it might, perhaps, be justified on a theory once held by Prof. Bower and sometimes known as that of the "primeval strobilus." On this view, the whole leafy plant represents a strobilus, once fertile throughout, but now largely sterilised, most of the sporophylls having become mere vegetative leaves. Dr Lashevsky makes no mention of any such theory, and we need not pursue the speculation further.

On the ordinary view that the leaves of Lycopods are what they appear to be—just vegetative leaves—there is certainly no reason why they should bear the relics of sporangiophores.

It may further be pointed out that in the sporophyll the ligule has no special relation to the sporangium. It is not connected with it, nor does the little strand of tracheides, which often runs up to the base of the ligule, in any way serve the sporangium.

The ligule, in fact, throughout the recent heterosporous Lycopods and those of their Carboniferous relations in which it has been demonstrated, is a remarkably constant organ, and never betrays any sign of reduction from something quite different.

This is not the place to enter on any general discussion of the question of the sporangiophore. As Dr Margaret Benson wrote in 1908: "It has been again and again suggested that we have in the Lycopodineous 'sporangium' a reduced structure which is homologous with the sporangiophore of the Sphenophyllales. The evidence in favour of this 'reduction hypothesis' is still very inadequate²." Dr Benson considered that the structure of her *Mazocarpon* and another somewhat similar fructification lent some support to the hypothesis of a reduced sporangiophore in Lycopods. Kidston and Lang, in the

¹ M. Benson, "The sporangiophore. A unit of structure in the Pteridophyta," *New Phytologist*, 7, p. 143, 1908.

light of their Rhynie discoveries, point out that "The sporangiophores would appear to represent the last persisting remains of the original leafless branch-systems of the Rhyniaceae." A closely similar suggestion had been made by Dr Benson in 1908 on less definite data. Kidston and Lang add that this interpretation, while directly applicable to the groups with evident sporangiophores applies "with greater difficulty and obscurity to the position of the sporangia of the Lycopodiales¹."

Thus the presence of a reduced sporangiophore in the Lycopods generally may be admitted as possible. But we cannot go further than this until evidence, more cogent than has hitherto been adduced, is available.

III. THE LIGULE OF CONIFERAE

Dr Lashevsky's third note is headed "On the Ligule of Coniferae (Pinaceae)," and is thus concerned with one of the most vexed questions of botanical morphology. It is an extraordinary fact that botanists have never yet been able to agree on an answer to the plain enquiry: "What is a Fir cone?"

At the end of his previous note Dr Lashevsky raised the question, whether the ligule on the bracts of Coniferae might not elucidate the significance of the ligule in Lycopodiales. In commencing the third note he refers again to his theory of the relation between the *Palaeostachya* type and that of *Lepidostrobus*.

But though the two contributions are thus closely connected, it must not be supposed that Dr Lashevsky regards the so-called ligule of certain Coniferae as homologous with the true ligule of heterosporous Lycopods. From the note alone one might imagine that this was intended, but in a letter dated October 29th, 1924, Dr Lashevsky agrees that there is no connection between the ligule of Coniferae and that of Lycopodiales, "but their evolutionary processes are alike." Thus, the case, on his view, is one of parallel development, not of affinity. In both groups the last rudiment of a reduced sporophyll appears as a "ligule," but the ligules are different.

The author traces the modification of the ovuliferous scale in some detail, from the Abietineae through the Taxodineae and Cupressineae to the Araucarineae, taking these groups in order as a reduction series.

¹ Kidston and Lang, "On Old Red Sandstone Plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part IV," *Trans. Royal Soc. Edinburgh*, 52, p. 850, 1921.

Throughout the series, as here interpreted, each scale of the female cone is composed of a lower part, the bract (*Deckschuppe* of the Germans), and an upper part, the ovuliferous scale (*Fruchtschuppe*), bearing the female organs.

The Abietineae have the bract and ovuliferous scale almost completely distinct. The relative dimensions of the two organs are variable within the family. In *Picea* and *Pinus* the bract is minute compared with the ovuliferous scale. The vascular bundles of the latter have inverted orientation of the wood and bast. The bundles of bract and scale run independently into the axis of the cone.

In the Taxodineae the bract and ovuliferous scale are almost completely coalescent. In *Cryptomeria*, for example, there is no definite boundary between the two. After the development of the ovules is complete the ovuliferous scale appears as a slight swelling on the base of the bract.

In Cupressineae the bract and ovuliferous scale are partially consolidated. In *Biota*, for example, they are closely united; the end of the ovuliferous scale appears as a slight swelling; it is sometimes like a ligule. The ovuliferous scale originates late, when the development of the female organs has long been finished. The bundles of the scale are joined to those of the bract.

The Araucarineae have the bract and ovuliferous scale completely coalescent. In *Araucaria Cunninghami* there is a typical ligule above the single ovule. There is a branch vascular supply to the ligule as well as to the ovule. In *Dammara (Agathis) australis*, only a slight swelling represents the ovuliferous scale, with a little depression in which the ovule is seated. The swelling has no vascular bundle.

Dr Lashevsky sums up the relations between bract and ovuliferous scale in the various groups as follows:

1. The ovuliferous scale constantly lags more and more behind the bract in its development (origin of the bract in autumn, of the ovuliferous scale in the following spring; development of the scale before or after that of the female organs).

2. Its vascular system shows a diminishing independence (less branching, earlier union and longer connection with the vascular system of the bract; departure from the main bundles first in the axis, then in the bract, and finally just below the insertion of the ovule).

There is a gradual reduction of the free part of the ovuliferous scale above the female organs (first it is larger than the bract, then smaller, then like a ligule, and finally only a slight swelling).

Thus the different groups show all stages of the gradual union of the two organs, bract and ovuliferous scale. The extreme limit is reached in *Agathis*, among the Araucarinceae. Here we have only a single bract, with one bundle and a little branch, and on the upper surface there is a megasporangium and a ligule. This, the author tells us, is a condition quite like what is found in the bract of fossil Lycopodiales. Thus he regards the Coniferous data as supporting his theory, already discussed, of the morphology of ligulate Lycopods. There is, undoubtedly, a certain analogy between the two interpretations, but both are highly hypothetical, and each must be judged on its own merits.

The following passage puts Dr Lashevsky's case plainly. "It is entirely admissible to suppose that among the Coniferae or their ancestors, as in fossil Lycopodiales, during the development of their fructifications a process of displacement of the fertile part into the axil of the sterile part took place; thus the ovuliferous scale and bract were originally attached independently to the cone-axis, in different whorls or spirally. The shifting of the ovuliferous scale into the bract-axil for its protection was the first step towards their union."

The author points out that no such changes were necessary in the case of the staminate cones, where the reproductive bodies formed are minute and are at once discharged.

Dr Lashevsky makes some reference to previous theories of the morphology of the female cone. It is unnecessary to follow him here, for the subject has been discussed often enough already—and without result!

The author regards his own theory as a vindication of a statement which he attributes to the great Robert Brown—that the ovuliferous scale is a leaf in the axil of a bract. I do not find that Robert Brown himself used this expression. He spoke of "a modified leaf" and an "open ovarium" as probably bearing the ovules in Coniferae¹; the phrase "*folium in axillâ folii*" was the weapon of his critics.

Still it is true that Dr Lashevsky's view is in accordance with that of the "*Botanicorum princeps*," for both interpret the ovule-bearing organ as a leaf—not as an emergence or an axillary shoot.

As we have seen, the author treats the female cones of Pinaceae, from *Pinus* to *Agathis*, as a reduction series.

¹ Robert Brown, "Character and description of *Kingia*...with observations on the female flower of Cycadeae and Coniferae." London, 1827. Reprinted in his *Miscellaneous Botanical Works*, Ray Society, see 1, pp. 458, 460, 1866.

The opposite view might be, and has been, maintained. In reality, we may be sure that there is no series either way, for the existing groups are, no doubt, the surviving terminals of branch-lines of evolution. Their history is lost; so far, the fossil Conifers have thrown little or no light on the evolution of the cone.

Dr Lashevsky's theory seems to be quite tenable, though it does not account for the inversion of the bundles commonly met with in the ovuliferous scale. There is no real impossibility in the conception of "*folium in axillâ folii*." Similar arrangements constantly occur or appear to occur in the Angiospermous flower, as the author points out.

It might be objected to the theory that no case can be cited where the two organs—bract and ovuliferous scale—are completely autonomous and inserted independently on the axis. Just as in the *Palaeostachya* case, the stages of fusion (*i.e.* the late stages) were missing, so conversely, in the case of Conifers, the stage of complete independence (the earlier condition assumed) appears to be missing.

It may be suggested that Dr Lashevsky might have found some support for his theory in the female fructification of *Cordaites* (*Cordaianthus*).

Here there are sterile bracts intermingled in the cone or catkin with the fertile sporophylls. This seems to be almost the condition which the author's interpretation requires as a starting point. The interpretation of *Cordaianthus* itself is open to discussion, and no one would suppose that it lay on the direct line of evolution of the Pinaceae. Still, the presence of sterile bracts in a Gymnospermous cone of Palaeozoic age would seem to be a fact of considerable significance from Dr Lashevsky's point of view.

The writer of these comments has himself inclined to Eichler's placental theory of the ovuliferous scale. This view may be held to imply that the Araucarian condition is primitive, more especially as the Araucarians are known to be an ancient race. It must, however, be admitted that in *Araucaria*, at any rate, the appearances strongly suggest a scale adherent to the bract, a condition favouring reduction rather than origination.

It may well be that the ovuliferous scale (whatever its origin) is a very old formation, and that on some lines of descent it has suffered reduction, while on others it has gained the upper hand over the bract.

The riddle of the Fir cone remains unanswered. Dr Lashevsky has made a gallant attempt to find a solution, and has, perhaps,

done as well as any of the numerous theorists who have gone before him.

At a time when Morphology is no longer a very popular branch of our science, Dr Lashevsky has done a service in bringing these questions once more before us. Working under the greatest difficulties, happily unknown to us in this country, he has shown admirable enthusiasm and no little ingenuity. We may hope that, as conditions become more favourable, he may find opportunities of testing his theoretical conclusions by original research.

ADDENDUM

Since the preceding pages were written, Dr Lashevsky has sent me a revised version of his notes.

In Note I, on the Ulodendroid scar, he cites a specimen of Renier's, which shows a dichotomous branch on the side of the stem opposite a Ulodendroid scar (see above, Sect. I). He now refers to the hypothesis of Carruthers (see p. 39), but without criticism.

In Note II, on the Ligule of Lycopodiales, the author now lays stress on the case of *Bothrostrobilus*. He considers the large ligule in that genus as a sign of the great antiquity of the *Bothrostrobilus* fructifications as compared with *Lepidostrobilus*. It may, however, be pointed out that the species (cone of *Bothrodendron mundum*) in which the ligule has been demonstrated, is no older than the Coal-Measure *Lepidostrobus*. In the species of *Bothrodendron* (*Cyclostigma*) from the Devonian, there is said to be no ligule¹.

The third note, "On the Ovuliferous Scale of the Coniferae," has grown to many times its original length. We can only notice a very few points in this long essay. The author attaches much importance to "marks of affinity" between Lycopods and Conifers, as justifying the application of comparative anatomical methods to these two groups.

He calls attention to the "poche" or sinus at the base of the ovuliferous scale in some species of *Abies*. It has a double epidermis, which he regards as "the strongest evidence of the former independence of the bract and the ovuliferous scale."

The anatomical details are considerably extended, and a much fuller résumé of previous opinion on the morphology of the cone is now given. The author's account is well up to date and covers the recent views of Jeffrey, Goebel, Wettstein and Kozot-Poljansky.

¹ See Gothan, *Potonié's Lehrbuch der Paläobotanik*, 2te Auflage, p. 188, 1921.

He scarcely, however, does justice to the placental hypothesis of Eichler, which many botanists accept as the most reasonable.

Towards the end, Dr Lashevsky makes a somewhat startling departure from his original interpretation, for he says: "We do not believe the ovuliferous scale to be a macrosporophyll. It does not bear the macrosporangia immediately on its surface." He now adopts the "wing" as the true macrosporophyll, homologous as he believes, with the microsporophyll or stamen. He upholds this interpretation, while admitting that the nature of the wing is quite different in different Conifers, and that in some it is altogether absent. To the present writer, the wing seems an inconstant and therefore a relatively unimportant organ; the new hypothesis can scarcely be regarded as strengthening the author's position.

In conclusion, certain points from Dr Lashevsky's rather extensive final summary may be noted.

The modifications to which the apparent complexity of the female strobilus are due appear, thanks to the influence of bad conditions and in order to protect the megaspores better. These modifications consist in the shortening of the axis and the contraction of the whole strobilus, and in the consequent shifting of some of the scales into the axils of others, with a greater or less degree of welding.

The ovuliferous scale is the equivalent of the bract; its situation in the axil of the latter is derivative, a result of displacement from a higher level.

The ligule of the bract is a relic of the apex of the ovuliferous scale. The two wings of the ovule constitute a scale (apparently a megasporophyll), having shifted into the axil of the bract.

The Abietineae are exceedingly ancient in regard to the structure of the female strobilus; it is impossible to connect the Abietineae with the Lycopods through the Araucarieae; the simple scales of these two groups are, in reality, compound.

The Lycopodiales and Araucarieae are final branches from apparently the same ancestor, and parallel in their development.

Thus Dr Lashevsky is an adherent of the theory of an affinity between Conifers and Lycopods, but on quite different grounds from those on which the somewhat similar conclusions of Professor Seward and others were based.

D. H. S.

OBSERVATIONS ON CLOVER ROT (*SCLEROTINIA TRIFOLIORUM* ERIKS.)

BY S. M. WADHAM, M.A.

(With 2 figures in the text)

DURING the period 1919-1923 work on this destructive fungus was carried out on the University Farm and in the Botanical Laboratory at Cambridge. The main objective was an economic one, but attractive side issues of a theoretical nature have been opened up. In the latter years it has become increasingly difficult for the writer to continue the field work and the present publication of results is necessary in order that that side of the problem may be taken up by others.

The disease, as seen from the farmer's point of view, has already been described (Amos, 1918(1); Boeker, 1919(3); Wolf and Cromwell, 1919(4)), but a brief recapitulation of its normal course seems desirable.

In the early autumn, after a spell of damp weather, flattish apothecia emerge above the surface of the soil in an old clover ley; they are pinkish-brown in colour and may reach 8-10 mm. in diameter¹. If the weather is fairly dry they continue to appear until the end of November. Nothing happens to the new clover fields for some time, but if the growth is a luxuriant one plants here and there begin to show signs of the disease in December; the outer leaves die off rapidly, assuming an olive-brown colour, a diffuse mycelium can be seen on the sides of the clover root-stock and on the adjacent soil²; more leaves die and frequently the whole plant is destroyed. The number of plants attacked increases, and in the meantime a search among the decaying tissues of the dead plants reveals the presence of numerous sclerotia; these lie dormant in the soil until the following autumn or later. Many leguminous crops are attacked. See Amos, 1918(1).

¹ Mr Amos has kindly supplied the dates on which he first observed apothecia in the years 1915-19; they are Oct. 30th, 6th, 20th, 11th, and Aug. 31st respectively.

² Mr Amos' earliest dates on which the mycelium appeared in quantity in the years 1917-19 are Nov. 14th, Dec. 9th and Dec. 5th respectively.

The Mycelium. This has been grown in the laboratory from spores, from sclerotia and from mycelium taken from the ground in the field. It can be easily cultivated on a large number of media. The fungus tolerates a wide range of acidity and alkalinity. It agrees in its general behaviour in culture with the description of the mycelium of *S. sclerotiorum* given by De Bary in 1886(2). On clover agar at 14–16° C. its rate of spread is somewhat less than that of *Cladosporium herbarum* and *Penicillium* sp. The hyphae vary in diameter from 5 μ to 12 μ , they are freely branched and show frequent anastomoses. Hyaline vacuoles are common when rapid growth is in progress. The rate of growth varies with the moisture in the medium; thus in a damp culture tube the mycelium is capable of crossing a space devoid of food substance 5 mm. in breadth between two blocks of nutrient material; this is a point of some practical importance, as in a clover field in autumn there is usually a dense growth of vegetation rendering the air moist at soil level; further, there is a good deal of plant detritus forming a suitable medium for growth, so that the fungus can and does spread over the ground as a thin, felted mycelium. It has already been stated that such mycelium collected from the soil has been transferred to tubes and has been shown to be capable of producing typical sclerotia. Cultural experiments indicate that there is little if any difference between the rates of growth of the mycelium when subjected to daylight and when kept in the dark.

Sclerotia. On a suitable substratum the mycelium spreads at first evenly and then it becomes locally denser, forming small white masses; drops of liquid are exuded, and sclerotia are gradually formed; these are at first olive-green on the outside, but they rapidly blacken. In the field they may be formed wherever there is an available space in or near the tissues of the dead host plant. In clover the commonest situation is between the shrunken central cylinder of the root-stock and the crushed epidermal and cortical tissues—here the sclerotia may be as large as 15 mm. by 3 mm.; the centre of the leaf petiole is another common situation, whilst on the finer roots sclerotia may be produced on the outside of the dead tissues in a crack in the soil. In bean plants deliberately infected with the fungus the hollows of the dead haulm are their usual position. Their shape is in all cases that of the cavity in which they are formed, or rounded if they are freely exposed.

Numerous experiments were carried out with sclerotia. They were found to absorb moisture from a damp atmosphere very readily, and

can be alternately moistened and dried without loss of vitality. A batch which had been subjected to this treatment in an incubator at 22° C. for five months was divided into three groups. The first was set under favourable conditions for the production of apothecial shoots; they did so within a week. The second was kept at 22° C. for a further five months and were then treated similarly with the same result. The third was heated in a drying oven at 80° C. for 80 hours and then set to "germinate"; they did so successfully. Specimens which had been collected in 1916 and stored in a dry corked bottle were in 1921 moistened and after superficial sterilisation placed on clover agar; they soon produced a typical mycelium which formed fresh sclerotia.

All live sclerotia will produce a mycelium if moistened and kept in a saturated atmosphere; *e.g.* on wet cotton wool they will produce a weft of hyphae on the surface. On a nutrient medium the mycelium gives rise to secondary sclerotia of normal appearance. The average time required for the production of sclerotia under these conditions may be gauged from the following observations. A sclerotial fragment was placed on nutrient agar in a tube on May 8th, the tube being kept at the laboratory temperature; it had produced fresh sclerotia by June 14th. One of these, transferred to a fresh tube, went through the process again by July 16th, the next crop was mature by August 25th, and the next was dry by October 2nd. There was no observable diminution in size of the sclerotia of successive crops.

There seems to be no reason why the sclerotia in the soil should not produce a mycelium which could, after growth on vegetable detritus, infect leguminous crops unless the competition of other micro-organisms in the soil is too severe for the mycelium of *Sclerotinia*. This is probably the case in the vast majority of instances; endeavours were made to infect clover plants growing in pots by burying sclerotia and clover crop at various depths, but the results were negative. It is, however, felt that the possibility is worthy of further examination.

Apothecia. When a sclerotium is kept continuously moist for a period it puts out one, or more frequently several, apothecial shoots. Numerous attempts have been made to ascertain the exact conditions under which they are produced, but success up to the present has been only partial; it is intended that this phase of the work, together with the cytology of the apothecium, shall be continued in the near future. The outsides of the sclerotia were sterilized before they were

set to "germinate" because bacteria, saprophytic fungi and mycophagous flies usually made their appearance when that process was omitted. The period of germination was usually found to be from 14-21 days, but it was so erratic that no general conclusions can be drawn. The most satisfactory substratum was wet cotton wool; in using it care had to be taken to obtain a flat wet surface, for sclerotia placed on raised patches usually failed to germinate, as did any which happened to fall into water-filled depressions.

The tip of each young apothecial shoot is usually concave and is invariably darker in colour than the lower portions. Branched and otherwise abnormal shoots sometimes occur. The shoots exhibit marked positive heliotropism. The development of the brownish tips into the flattened apothecium was found to be erratic; observation showed that it seldom if ever took place in diffuse light, the greatest number of successful cultures being found among those which were placed in a sunlit window: opened apothecia are at first flat and then, as they broaden, they often become depressed in the centre. The duration of an apothecium in the field varies to a certain extent with the weather; continuous rain causes their early decay. In the laboratory they have been found to last for a month. No attempt will here be made to give an account of the cytology of the species as the work is incomplete and it is hoped to continue it in the future.

Field Behaviour and Remedial Measures

Plates of nutrient media were exposed to the air in a field where apothecia were prevalent. They were placed at ground level and also on the tops of stakes one metre high. Some of the plates developed the type mycelium and formed sclerotia, although the number of colonies of bacteria and fungi other than *Sclerotinia* was naturally large. Plants on whose leaves pure water containing spores was placed invariably failed to be infected unless the tissues of the leaf were damaged, or a small amount of nutrient solution was added to the water. It seems clear, therefore, that a saprophytic mycelium must first be developed before living plants can be attacked. The suggestion that this mycelium originates on the decaying clover leaves on the surface of the ground and then spreads slowly to the clover plants seems to be a reasonable one; but the possibility that sclerotia in the soil may occasionally give rise to a mycelium directly must not be disregarded. The way in which the disease spreads from plant to plant was studied by means of periodic observations on small selected plots in a field of clover and rye grass. Unfortunately, the

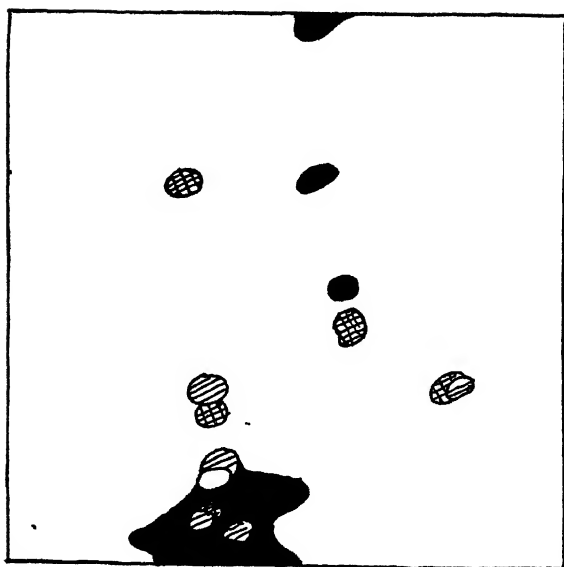


Fig. 1

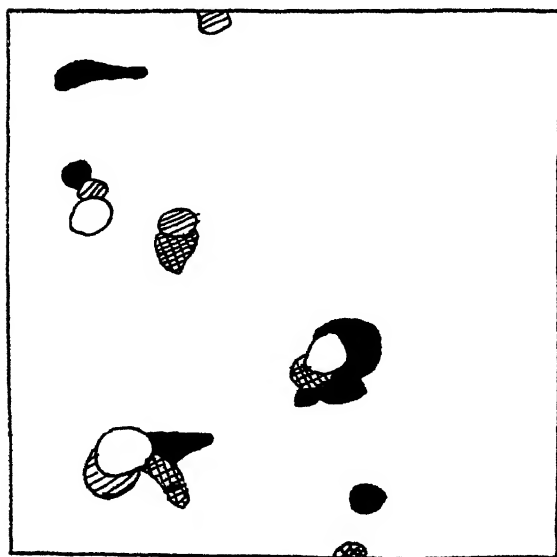


Fig. 2

two seasons during which these observations were made were marked by relatively long periods of weather which were not favourable to the development of the mycelium; so that the disease did not assume epidemic proportions.

In 1919-20 the method was tested, and in 1920-21 twenty-one plots were kept under observation, some of these being treated with materials which it was hoped would check the growth of the mycelium; others were kept as controls. The figure shows the results obtained for some of these control plots; it is noticeable that whereas in many cases the attack starting from centres of infection spread slowly, in others the fungus made no appreciable advance: further, it is to be noted that cases of infection which are apparently new appear as late in the season as March. It was unfortunate that the crop was not pure red clover and also not a very heavy one.

In 1920-21 some of the areas were located on a part of the field which had been folded and grazed by sheep and on which the clover plants were naturally small; there was a definite contrast in the amount of disease on the "sheeped" and "unsheeped" zones. This may be explained by the difficulty which the mycelium experiences in crossing a patch of soil which is not sheltered by surrounding herbage and on which the superficial detritus has largely been destroyed by the sheep.

It is clear from the experience gained in this way that any method of reducing the density of the plants or of destroying the layer of detritus on the soil will lead to a decrease in the rate at which the fungus can spread. Further, the mycelium is the only stage in the life-cycle of the disease at which there is any chance of dealing with it satisfactorily. The sclerotia are exceedingly resistant and the apothecia are small, inconspicuous, and appear during a fairly long period. The hope that a dressing of fungicide applied to the soil and its

EXPLANATION TO TEXT-FIGURES

Figs. 1 and 2. Two plots, each of an area of 4 square metres, showing location of new areas bearing plants infected with the disease at various dates during the winter.

Enclosed areas without hatching show position of plants attacked before 20 Dec. 1920.

Enclosed areas with single hatching show position of plants attacked before 1 Feb. 1921.

Enclosed areas with double hatching show position of plants attacked before 15 Feb. 1921.

Enclosed areas in black show position of plants attacked before 21 Mar. 1921.

detritus at a time when the ascospores were being scattered might prevent their further development has at present proved vain¹.

The writer is greatly indebted to Mr A. Amos and Mr F. T. Brooks for help in the work described; the former as a result of his long practical experience of the disease has been able to assist and advise in many ways, whilst the latter has been a continual guide in the laboratory investigations.

SUMMARY

The mycelium of the fungus is readily cultivated and forms sclerotia on various media.

The sclerotia have no definite shape, they are exceedingly resistant to changes of temperature and to desiccation. They readily give rise to a mycelium.

Exact control of the development of apothecial shoots has not yet been obtained. These shoots are positively heliotropic; they do not open to give apothecia unless they are brightly illuminated. The spores give rise to a mycelium which is at first saprophytic and then becomes facultatively parasitic. The spores appear to germinate on vegetable detritus on the soil; the mycelium spreads over the surface of the ground, its rate depending on the atmospheric conditions at the soil level: these in turn are greatly affected by the density of the crop.

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¹ Three of the fungicides used were 2 per cent. solutions of cresol, ferrous sulphate and copper sulphate respectively; they were applied at the rate of 300 c.c. per square metre. In no case did there appear to be any check to the spread of the mycelium; the treated plots developed new infections. In 1921-2 naphthalene, lime-sulphur, "Blighty Mixture," and "Vaporite" were also tried, but the only positive result was the death of the clover in certain cases.

THE FIRST SUGAR OF PHOTOSYNTHESIS AND THE RÔLE OF CANE SUGAR IN THE PLANT

A REPLY TO PROFESSOR J. H. PRIESTLEY

By JOHN PARKIN

At the present time it may be stated with some truth that the facts, or shall we say the presumed facts, relating to the carbohydrates of plants are not exactly in harmony with those respecting the chemistry of this class of carbon compound. That is to say, the plant at times appears to deal with carbohydrates in a manner contrary to laboratory experience. The chemist may reply "So much the worse for your botanical facts!" Such an imaginary gibe may or may not be merited. Time will show. Professor Priestley, realising this apparent discrepancy, has recently¹, in an ingenious way, endeavoured to bring about a reconciliation. A critical review of his paper with some general comments on the rôle of cane sugar (sucrose) in plants may not be unwelcome from one who some years ago took an active part in the difficult work of estimating sugars in foliage leaves. To me, and apparently also to him, the difficulty of squaring matters with the chemist centres round the origin of cane sugar in the plant. This carbohydrate has an annoying habit of turning up where one might least expect it on *a priori* or chemical grounds!

In accordance with the theory of photosynthesis usually adopted, viz. that of Baeyer, still unproven, the appearance of cane sugar in the leaf as the result of carbon-assimilation is explained somewhat as follows. The formaldehyde is polymerised to hexose, presumably glucose (dextrose). Part of this is changed into fructose (levulose), and then the two hexoses combine to form the disaccharide, sucrose. To account for the transformation in the leaf of part of the glucose into fructose, use is made of the chemical fact that a mixture of hexoses, viz. glucose, fructose and mannose, appears in a solution of any one of them, when this is rendered alkaline. Mannose then should also be found in the leaf. So far it has not been detected in leaves which have been especially examined for their sugars. It is a hexose easily recognised on account of its insoluble hydrazone. The formation of sucrose from glucose and fructose is considered to be brought about by the synthetic (reversible) action of the enzyme, invertase. This,

¹ Priestley, *New Phytologist*, 23, No. 5, p. 255, Dec. 1924.

as Priestley acknowledges¹, has not yet been demonstrated. Regarding the reverse change—the hydrolysis of cane sugar into the two hexoses, both botanists and chemists are agreed that this is, or can be, brought about in the plant by the invertase. The foregoing speculation regarding the manner of formation of cane sugar in the leaf through photosynthesis may in a measure be considered to satisfy the chemist. It is well at this stage to recognise that sucrose still eludes synthesis in the laboratory. When this is accomplished perhaps some new light may be shed on its mode of origin in the plant.

Though Prof. Priestley favours the above view rather than the one holding sucrose as the first sugar of photosynthesis, he closes this part of his paper with the following somewhat perplexing passage¹: "On consideration, however, there seems to the present writer to be grounds for thinking that cane sugar formation in the plant usually proceeds upon other lines and that its formation from the hexoses first formed in photosynthesis is far less direct." Now this passage is concerned with his new hypothesis which suggests meristematic tissue as a source of cane sugar. Though this novel idea will be dealt with later, it is puzzling here to understand how he can explain in this way the cane sugar present in the foliage leaf. He considers, as the above extract shows, that its formation from the hexoses first formed in photosynthesis is usually far less direct than on the supposition that it results directly from the combination of glucose and fructose. But there is no meristematic tissue in the normal adult assimilating leaf, and the cane sugar present cannot well have been conveyed thither from other regions of the plant, as it increases greatly in quantity in detached insulated leaves. I may be misunderstanding the author here; but at any rate I fail to see how his new theory can account for the cane sugar of the foliage leaf.

The view put forward that the cane sugar of the leaf arises through the condensation of the hexoses formed in photosynthesis is not only in keeping with the formaldehyde theory, but rests also on a certain amount of experimental evidence. Priestley brings forward the latest support in the shape of some very interesting observations of Weevers². As these are recorded in a not very accessible journal, he very kindly posted me his copy. On its perusal I feel, Agrippa-like, almost persuaded! Whether or no *free* hexose actually precedes the formation of sucrose in photosynthesis, the writer is still of the

¹ Priestley, *loc. cit.* p. 258, para. 1.

² Priestley, *loc. cit.* p. 258, para. 2.

opinion that the bulk of the hexose sugars found in the foliage leaf has come from the sucrose through inversion.

In the light of our knowledge of the chemistry of the carbohydrates, taking glucose as the initial and starch as the final product, one might have expected the disaccharide of the leaf to have assumed the form of maltose; but this is not so. Consequently on the glucose theory both starch and sucrose are looked upon as arising in the leaf from hexose sugar independently of one another. But there is a fair amount of evidence to suggest that in plant metabolism these two carbohydrates are intimately connected, so much so as to warrant calling cane sugar the precursor of starch. In feeding destarched leaves with sugar solutions sucrose proves a better starch-former than invert sugar. This suggests either that the sucrose is transformed into starch directly without inversion, or that on hydrolysis the hexoses appearing in the "nascent" state are more readily transformed into starch than when they are presented in the more stable form in solutions.

Turning now to the origin of cane sugar in parts of the plant other than the green assimilating organs, it may almost be taken as proved, considering the evidence available, that whenever reserve-carbohydrates are being hydrolysed for the purposes of growth cane sugar quickly makes its appearance. This is true both in the germination of seeds and in the sprouting of bulbs, tubers, etc., and is irrespective of the nature of the reserve-carbohydrate, which may be starch, inulin, mannosan, galactosan or some mixed polysaccharide. This seems also to be the case when the reserve is in the form of fat (oil) instead of carbohydrate. It is not intended to infer that there is a direct transformation of these carbohydrate-reserves into sucrose. The evidence points the other way, viz. that each is hydrolysed down by its respective enzyme into the corresponding sugar, and it is this that is transformed into sucrose. It would appear, then, that no matter what may be the character of the non-nitrogenous reserve, the plant for the purpose of growth turns this as quickly as possible into cane sugar.

This aspect of the matter would seem also to apply to the phenomenon known as the "rise of sap," shown by trees in early spring. In the sap of trees which "bleed" readily on being wounded, *e.g.* vine, maple and birch, it has been ascertained that the sugar present consists almost wholly of sucrose. The little hexose that may be found is considered to have arisen from the cane sugar, and Priestley thinks this view inevitable in the case of the sycamore¹, and I agree.

¹ Priestley, *loc. cit.* p. 258, para. 3.

The sucrose in the sap of these trees is generally regarded as derived from the starch stored in the wood-parenchyma. Just as in the case of sprouting seeds, tubers, etc., there is no need to infer a direct change of starch into sucrose. The former is probably broken down in the usual way, and either the maltose or the glucose is transformed into cane sugar on its way through to the xylem vessels. Prof. Priestley would appear to make unnecessary difficulty here. To quote¹: "If, then, cane sugar is to be formed [from the starch], it would seem necessary from the chemical standpoint to obtain first fructose from the glucose and then a synthesis of cane sugar from these two hexoses. Clearly this point of view clashes with our own observations and those of Annett as to the relative order of appearance of cane sugar and hexoses in the xylem sap." But there is no need to regard the synthesis of the cane sugar as taking place in the xylem vessels themselves. This may, as suggested above, have come about earlier. Consequently one would not expect to find up-grade (pre-sucrose) hexose in the xylem sap.

He now proceeds to bring forward further reasons for challenging the "assumption that the cane sugar in the xylem sap arises from the starch contained in tissues in or near the xylem"²; and from the bottom of this paragraph it is fairly clear that what he intends to combat is that the starch in the wood-parenchyma of a certain part of the stem is responsible for the cane sugar found at this level. Presumably the sap, and so the dissolved sucrose, is moving in the vessels, at any rate when conditions are favourable, so it may quite well happen that both cane sugar and starch may be found in abundance side by side at a certain level of the stem, since the sucrose may have arisen from starch originally stored at a lower or different level. Some such reasoning may partly explain Butler, Smith and Curry's conclusion, mentioned by Priestley, to the effect that in the apple tree the concentration of the cane sugar is quite independent of the distribution of the starch.

The ringing experiments of Curtis are quoted in support of the view that the cane sugar of the xylem vessels is independent of the starch of the wood-parenchyma. Apparently we are to believe that this starch when it disappears travels as glucose and possibly also as maltose in the phloem; whereas the cane sugar from an independent source uses the xylem vessels for its conveyance. In endeavouring to elucidate the puzzling observations of Curtis, it seems to me he

¹ Priestley, *loc. cit.* p. 259, para. 1.

² Priestley, *loc. cit.* p. 259, para. 2.

adds to our difficulty of understanding how carbohydrates travel in the vascular plant. It may, of course, happen that there is more than one kind of channel for their translocation. The upholders of the phloem and xylem views may both be right; but this is not very satisfying in the present state of our knowledge.

Some more puzzling data from a quite recent (1924) paper by Ahrns are now brought forward. Contrary to expectation a wilting leaf is proved to contain more sugar and less starch than a control leaf with full water supply. A suggestion is offered, but it may be wide of the mark. May not the wilting have resulted in bringing the enzymes into closer association with the starch and thus led to greater hydrolysis, presuming the presence of sufficient water to permit of this? Ahrns' experiments, to the writer, would seem to favour rather than to weaken the view that cane sugar and starch are closely connected in plant metabolism. He finds that as the starch diminishes the sucrose and hexoses combined proportionally increase and *vice versa*. How can the cane sugar be accounted for without inferring some intimate connection between these two carbohydrates? It is not necessary, as already pointed out, to assume that "cane sugar arises by a simple process of hydrolysis from starch¹." It may be broken down in the usual manner and the sugar or sugars resulting therefrom transformed into sucrose by some as yet unknown synthetic agency.

Finally, we come to Prof. Priestley's new theory which, using largely his own words, states that cane sugar can arise "as a secondary product in the metabolism of [the meristematic] cell, being subsequently occasionally released from these cells as they vacuolate and differentiate²." Or, again, he is of the opinion that "the cane sugar widespread in plant tissues but not concentrated in amount as a storage product arises as a secondary product, formed first of all during the complicated metabolism associated with protoplasmic construction and therefore present in the growing cell³." He has been led to this, one might almost say startling, speculation by the interesting discovery of cane sugar in the growing root tip away from any starch. In the bean it would seem that sucrose is recognisable "in the last two millimetres of the germinating root tip, in the region where meristem cells are vacuolating and differentiating and is not present until the process of growth and differentiation has commenced after the beans have been soaked in water and germinated⁴."

¹ Priestley, *loc. cit.* p. 260, para. 3

² Priestley, *loc. cit.* p. 264, last para.

³ Priestley, *loc. cit.* p. 262, para. 4.

⁴ Priestley, *loc. cit.* p. 263, para. 2.

This research is still proceeding and we await keenly further details. The detection of cane sugar in these meristematic regions of the bean fits in with the writer's preconceived opinions; but only on the supposition that the sucrose has travelled thither from the cotyledons. If it can be conclusively proved that it cannot have arisen from carbohydrate stored in the cotyledons, then it must be admitted that Prof. Priestley has grounds for his theory. Till then my general opinion is that carbohydrate travels in the form of cane sugar to the meristematic regions and is there inverted as it is required for growth and respiration. It is just possible, though on chemical grounds unlikely, that cane sugar may to some extent be utilised directly in growth; but doubtlessly it is inverted first before it can serve for respiration. Though Priestley's theory may apply to the origin of sucrose in non-assimilating organs, it fails, as already pointed out, to account for the cane sugar of the foliage leaf.

It would be going beyond the scope of this article to comment further on the vexed question as to the channel or channels by which carbohydrates travel in the vascular plant. But a few words may be allowed as to the form the carbohydrate assumes in its translocation. Surely there can be little doubt but that in spring time sugar travels in the xylem vessels of trees in the form of sucrose. How otherwise can the fact be explained that in "bleeding" trees the sugar in the sap is almost wholly composed of sucrose? On the other hand, in the foliage-leaf-work the analyses point to the carbohydrate formed in photosynthesis passing out largely as hexose sugar. This appears to be the case both for the beet (mangold) and the snowdrop leaf. In these two instances the sugar is descending to be stored. Can it be that the carbohydrate travels largely in the form of cane sugar when needed for immediate growth and in the form of hexose when required for storage purposes? This is a mere conjecture, which does not altogether commend itself to the writer, but is here given for what it is worth. It is well always to bear in mind the possibility that some of the hexose sugar found in manipulated plant extracts may not have been there originally in the tissues. Cane sugar is so readily inverted both by acids and its own enzyme. However, in the careful work on the mangold leaf by the Rothamsted investigators (Davis, Daish and Sawyer) the chance of much inversion during manipulation would appear to be extremely small.

To discuss at length the possibly different rôles played in plant metabolism by the two hexoses (glucose and fructose) is premature,

since Davis¹ has shown that no reliance can be placed on the figures given in past analyses for these two sugars. The evidence, such as it is, appears to point to the glucose being more readily used in respiration and to the fructose being of more service in growth. The one then for katabolism and the other for anabolism. It is, I imagine, a fact that fructose (hence its name) predominates over glucose in most ripe fruits. This supports the view of glucose being more easily consumed in respiration. But the excess of fructose might conceivably be explained as an adaptation. It is the sweetest of the sugars, and thus may have been selected to accumulate in preference to the glucose.

A glance at the distribution of cane sugar, as far as we know it, in the plant kingdom is instructive. From the special researches of Schulze and Frankfurt, Bourquelot and Kylin, as well as incidentally from many other investigations, it may be taken almost for granted that cane sugar is universally present in Flowering Plants. It would, however, be interesting to know the nature of the carbohydrates and their distribution in the parasitic and saprophytic chlorophyll-free Angiosperms. Cane sugar has been shown to be present in the Conifers and the Vascular Cryptogams. It has been found to occur, even in abundance, in Mosses². The Algae largely await investigation. The writer is unaware of a clear demonstration of cane sugar in the Green Algae, but then they have hardly been examined. Kylin³ investigated the carbohydrates of certain of the Brown Algae and found glucose and fructose, but failed to demonstrate the presence of sucrose. The occurrence of fructose is suggestive of that of sucrose as well. Atkins⁴ stated in 1916 that as yet there is no evidence of sucrose in the Red Algae. Since then Haas and Russell-Wells⁵ have revealed its presence in Carrageen (*Chondrus crispus*). If the Red and probably the Brown Algae contain sucrose it is hardly likely that the Green do not. As regards the Fungi it is fairly clear that they do not contain sucrose. Another disaccharide, trehalose, appears to take its place. It is of interest to note that both sucrose and trehalose are non-reducing sugars. Unlike such disaccharides as maltose and lactose they do not reduce Fehling's solution or form osazones. The Blue-green Algae, as far as the writer can ascertain, have not been examined for their sugars. Apparently like fungi they contain glycogen in place of starch.

¹ Davis, *Jour. Agric. Science*, 7, p. 327, 1916

² Mason, *Proc. Roy. Dublin Soc.* 1915.

³ Kylin, *Zeit. Physiol. Chem.* 83, p. 171, 1913.

⁴ Atkins, *Some Recent Researches in Plant Physiology*, London, 1916.

⁵ Haas and Russell-Wells, *Biochem. Journ.* 16, p. 572, 1922.

The point of entry of cane sugar in the evolution of the plant-kingdom may thus be one of some importance. A thorough investigation of the Algae might solve the problem. From the meagre data we at present possess it looks as if the sucrose-line might be drawn between the green and blue-green algae. The animal kingdom, as far as we know, is devoid of cane sugar. In the alimentary canal it is inverted and then utilised; but if introduced directly into the blood it is inert. The two kingdoms probably diverged before cane sugar had made its appearance. The animal tissues seem to be singularly lacking in disaccharides; but there is one conspicuous exception, viz. the mammary gland of mammals. In milk, its secretion, the carbohydrate throughout this group of animals takes the form of lactose. According to the chemistry of the sugars one would have expected a disaccharide more after the style of maltose, viz. one hydrolysing to glucose only and not one affording galactose as well—a hexose which does not even conform to the enolic theory respecting glucose. Sugar is supposed to reach the mammary gland in the blood stream as glucose, previously stored as glycogen, and to be secreted in the milk as lactose. Can we not draw a rough analogy between the sequence

glycogen \rightleftharpoons hexose \rightleftharpoons lactose
and that of starch \rightleftharpoons hexose \rightleftharpoons sucrose?

Cane sugar, then, we have reasons to believe, is peculiar to the Plant Kingdom. That it plays a necessary and very important part in the metabolism of, at any rate, the Higher Plants can hardly be doubted. It may possibly have arisen at the time when the chloroplast and the starch grain were differentiated. This of course is pure speculation.

In conclusion, let us express our indebtedness to Prof. Priestley for re-opening the question of the rôle of this sugar in the plant by challenging with a new theory present opinions. Our thoughts have thus been focussed once more on carbohydrate metabolism, and one hopes not only the thoughts but the actions also of some of our younger botanists, physiologically inclined, may be turned in this direction. Carbohydrate research in plants by quantitative methods is tedious, difficult and laborious, but the reward should be proportionally great. A large number of estimations made under identical conditions with reasonable precautions may be more likely to give reliable results than a few only carried out with meticulous care. In dealing with plant extracts in analysis it is so easy, while straining at the gnat, to swallow the camel.

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ÜBER DIE WASSERSTOFFIONENKONZENTRATION [H⁺] ALS DETERMINATIONSFAKTOR PHYSIOLOGISCHER GEWEBEGESCHEHEN IN DER SEKUNDÄREN RINDE DER PFLANZEN

VON DR. H. PFEIFFER, Bremen

(With 5 figures in the text)

I. ÜBER DIE DETERMINATION KRISTALLINISCHER AUSFALLUNG VON CALCIUMOXALAT IN PFLANZLICHEN RINDENZELLEN DURCH DIE [H⁺].

Es liegt nicht in meiner Absicht, hier ein historisches Bild zu entwerfen von der allmählichen Entwicklung unserer Kenntnisse über den angedeuteten Gegenstand. Einen derartigen Überblick anzuführen mag einer zusammenhängenden Darstellung vorbehalten bleiben, wie sie vielleicht in Linsbauers *Handbuch der Pflanzen-anatomie* noch zu erwarten sein wird. Bei der Zielsetzung wirkten mehrere Gründe bestimmend ein. Schwerwiegend war darunter besonders derjenige, dass die Bildungen aus Calciumoxalat zu den am meisten verbreiteten Aschenbestandteilen zählen und am häufigsten in der lebenden Pflanze auskristallisiert vorgefunden werden. Wegen dieses Vorkommens berührte unsere Kenntnis von ihnen meine angefangenen Untersuchungen der sekundären Rinde der Pflanzen, deren Behandlung für Linsbauers *Handbuch* mir obliegt.

Den *Begriff der Determination* wollen wir hier im weitesten Sinne fassen. Es wird darunter also die Summe aller zureichenden Komplementärbedingungen verstanden, auf die ein (organisches) Gewebegeschehen zurückführbar ist. Es handelt sich somit sowohl um Determinationsbedingungen s. str., als auch um die Realisationsfaktoren im Sinne einer streng definierenden Entwicklungsmechanik der Pflanzengewebe (Pfeiffer, 1925 b).

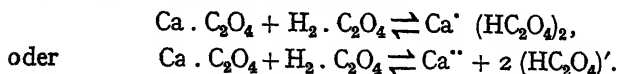
Die Frage nach den zureichenden Komplementärbedingungen für die Auskristallisierung von Calciumoxalat lässt sich sogleich in zwei subordinierte Probleme teilen, deren Beantwortung von uns schritt-

weise vorzunehmen ist. Sodann dürfen weitere, bei der Untersuchung aufgetauchte Fragestellungen kurz erörtert werden.

1. *Über die Relation der $[H^+]$ zur Auskristallisierung von Calciumoxalat im allgemeinen.*

Bei der Kristallisation aus Lösungen der Pflanzenzellen wird die Temperaturverschiebung wohl nur selten die zureichende Komplementärbedingung darstellen. Auch Druckänderungen werden hier nur in sehr untergeordnetem Grade für die Einleitung einer Kristallisation von Bedeutung werden. Ob ein fester Stoff aus einer Lösung kristallisiert oder nicht, hängt dann sicher besonders von seiner Löslichkeit in dem betreffenden Lösungsmittel und von der gelösten Menge der Substanz ab. Notwendig ist aber bekanntlich das Eintreten einer Kristallisation selbst aus einer gesättigten Lösung nicht. Ob sich die Löslichkeitsbeeinflussung in einer Vermehrung oder Verminderung der Löslichkeit ausdrückt, hängt nach A. Thiel (p. 1036 sq.) z. Bsp. davon ab, ob durch den Zusatz eine Über- oder Unterschreitung des Löslichkeitsproduktes herbeigeführt wird. Es liegt nahe, dass hier der Plan entsteht, die Einwirkung vorhandener freier H-Ionen zur Einleitung des Kristallisationsprozesses zu untersuchen. Man könnte sich die *Bedeutung freier H-Ionen* etwa ähnlich wie die auftretender Keime oder Richtungskörper in einer auskristallisierenden Lösung denken. In der Literatur ist übrigens bekannt, dass sich die Löslichkeit von Salzen schwacher, organischer Säuren zur $[H^+]$ proportional verhält [Michaelis, Kolthoff]¹. Theoretisch ist auch leicht einzusehen, dass die Löslichkeit mit steigender $[H^+]$ zunehmen wird. Als ein biologisches Beispiel wurde von Rona und Takahashi bereits das $Ca \cdot CO_3$ untersucht. Im Anschluss an die von Michaelis (p. 70 sq.) versuchte, abgeänderte Ableitung ihres Resultates kann man auch für das $Ca \cdot C_2O_4$ zu einem mathematisch definierten Ergebnis über die *Einwirkung der $[H^+]$ auf die Kristallisation* gelangen.

Die partielle Löslichkeit der Molekülgattung $Ca \cdot C_2O_4$ sei unabhängig von der $[H^+] = \lambda$; die totale Löslichkeit aber $\Lambda = \lambda + [Ca^{''}]$, worin $[Ca^{''}]$ diejenige Konzentration der Ca-Ionen darstellt, die sich mit der Konzentration λ der Molekülgattung $Ca \cdot C_2O_4$ im Gleichgewicht befindet. In der Lösung ist nun Gleichgewicht zwischen Calciummono- und Calciumbioxalat:



¹ Echte Neutralsalze (NaCl, KNO₃ usw.) dürften in ihrer Löslichkeit trotzdem kaum von der $[H^+]$ abhängig sein.

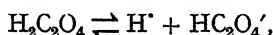
Daraus ergibt sich als Dissoziationskonstante:

$$k_1 = \frac{[Ca \cdot C_2O_4] \cdot [H_2 \cdot C_2O_4]}{[Ca^{..}] \cdot [HC_2O_4]_2} \dots\dots(1).$$

Zur Vereinfachung der Gleichung (1) bedenke man, dass:

I. in der Lösung $Ca \cdot C_2O_4 = \lambda \dots\dots(2).$

II. die Oxalsäure auch anders dissoziiert sein kann, nämlich:



woraus sich unter Benutzung der Dissoziationskonstanten ergibt:

$$k_2 \cdot [H^+] = \frac{[H_2C_2O_4]}{[HC_2O_4']} \dots\dots(3).$$

Durch Substitution der Resultate (2) und (3) in Gleichung (1) erhält man

$$k_1 = \frac{\lambda \cdot k_2 \cdot [H^+]}{[Ca^{..}] \cdot [HC_2O_4']}$$

oder (aufgelöst nach $[Ca^{..}]$):

$$[Ca^{..}] = \frac{\lambda \cdot k_2 \cdot [H^+]}{k_1 [HC_2O_4']} \dots\dots(4).$$

Als Endresultat für die totale Löslichkeit Λ ergibt sich also:

$$\Lambda = \lambda + \frac{\lambda k_2}{k_1} \cdot \frac{[H^+]}{[HC_2O_4']} \dots\dots(5).$$

Man sieht aus der Gleichung (5), dass die *Löslichkeit des $Ca \cdot C_2O_4$ nicht nur von der $[H']$, sondern auch von der Quantität der in Lösung befindlichen Bioxalat-Ionen¹* abhängt, mögen diese nun dem Bodenkörper entstammen oder künstlich als $NaHC_2O_4$ der Lösung zugefügt

¹ Welchen Einfluss die Salzquantität in der Zelle ausübt, ergibt sich durch mathematische Ableitung leicht wie folgt:

Ist x die Menge des in der Zelle gelösten $Ca \cdot C_2O_4$ zur Zeit t und also x_0 die ursprünglich vorhanden gewesene Quantität, so geht die Auskristallisierung nach der Formel:

$$x = x_0 \cdot e^{-\lambda t} \dots\dots(a)$$

vor sich, worin λ die Kristallisationskonstante darstellt. Die durch Gleichung (a) definierte Funktion $x = \phi(t)$ ist verschieden je nach dem Werte, welcher der Konstanten x_0 zugeschrieben wird. Um die allen diesen Funktionen gemeinsame Eigenschaft zu finden, wird die Gleichung (a) nach t differenziert:

$$\frac{dx}{dt} = -\lambda \cdot x_0 \cdot e^{-\lambda t} \dots\dots(b).$$

Eliminiert man nun noch aus den Gleichungen (a) und (b) die in jedem Spezialfalle verschiedene Grösse x_0 , so resultiert leicht:

$$\frac{dx}{dt} = -\lambda x \dots\dots(c).$$

Indem man den Bruch $\frac{-dx}{dt}$ der Kristallisationsgeschwindigkeit gleichsetzen kann, findet man die Geschwindigkeit der Auskristallisierung der jeweilig noch vorhandenen Quantität von $Ca \cdot C_2O_4$ proportional.

sein. Die hier jetzt unternommene Ableitung gilt für die Konzentration beider. Denken wir beispielsweise an das Salz, so löst es sich im Zellwasser auf und verteilt sich über das ganze Lumen der Zelle. Dabei wird die Verteilung, wenigstens anfangs, ungleichmässig sein. Bezeichnet man den Abstand einer gewissen Schicht vom Boden der Zelle mit x , so muss die Konzentration eine Funktion von x sein und mit wachsendem x abnehmen. Bei konstant gehaltenem x wird die Konzentration ρ eine Funktion der Zeit t sein, d.h. ρ ist eine Funktion der Variablen x und t . Das Salz muss fortwährend von Stellen höherer Konzentration zu solchen niederer hinüberdiffundieren. Nach der Diffusionstheorie (A. Thiel) dürfen wir annehmen, dass die durch einen bestimmten Zylinderquerschnitt pro Flächen- und Zeiteinheit wandernde Quantität dem an der Stelle herrschenden Konzentrationsgefälle proportional ist, also—wenn D als konstanter Diffusionsquotient¹ auftritt—gleich: $-D \cdot \frac{d\rho}{dx}$. Um die Beziehungen zwischen

der zeitlichen und räumlichen Änderung von ρ aufzudecken, denke man sich durch den Zylinder einer isodiametrischen Parenchymzelle zwei benachbarte Querschnitte, x_0 und $x_0 + \Delta x$, gelegt. Man erhält somit als die durch die beiden Querschnitte wandernden Quantitäten:

$$(I) -D \cdot \left[\frac{d\rho}{dx} \right]_{x_0}; \quad (II) -D \cdot \left[\frac{d\rho}{dx} \right]_{x_0 + \Delta x}.$$

In dem kleinen Zylinder ($x_0, x_0 + \Delta x$) kommt als Konzentrationserhöhung in Frage die Differenz:

$$-D \cdot \left[\frac{d\rho}{dx} \right]_{x_0} - \left(-D \cdot \left[\frac{d\rho}{dx} \right]_{x_0 + \Delta x} \right) \quad \dots\dots(6).$$

Setzt man den Flächeninhalt des Zylinderquerschnittes mit Q ein und den Mittelwert der Konzentration im Zylinder ($x_0, x_0 + \Delta x$) mit $[\rho]_{x_0 + \frac{1}{2}\Delta x}$, so erhält man die im Zylinder enthaltene Salzmenge $[\rho]_{x_0 + \frac{1}{2}\Delta x} \cdot Q \cdot \Delta x$, und es ist:

$$\frac{d}{dt} ([\rho]_{x_0 + \frac{1}{2}\Delta x} \cdot Q \cdot \Delta x) = Q \cdot D \cdot \left[\left(\frac{d\rho}{dx} \right)_{x_0 + \Delta x} - \left(\frac{d\rho}{dx} \right)_{x_0} \right] \quad \dots\dots(7).$$

Da nun Q und Δx in Hinsicht auf t konstant sind, so ergibt sich vereinfacht:

$$\frac{d}{dt} \cdot (\rho)_{x_0 + \frac{1}{2}\Delta x} = D \cdot \frac{\left(\frac{d\rho}{dx} \right)_{x_0 + \Delta x} - \left(\frac{d\rho}{dx} \right)_{x_0}}{\Delta x} \quad \dots\dots(8).$$

¹ Dadurch, dass das Konzentrationsgefälle gleich dem negativ gedachten Quotienten $\frac{d\rho}{dx}$ genommen wird, bekommt die rechte Seite der Gleichung negatives Vorzeichen.

Indem man in Gleichung (8) Δx gegen 0 konvergieren lässt, resultiert:

$$\frac{d\rho}{dx} = D \cdot \frac{d^2\rho}{dx^2} \quad \dots\dots(9).$$

Statt diese partielle Differentialgleichung zweiter Ordnung zu integrieren, möge man—unter der speziellen Annahme, dass es sich um eine stationäre Diffusion durch die Zellflüssigkeit handelt—die Gleichung (9) auf eine gewöhnliche Differentialgleichung reduzieren. Ferner ist anzunehmen, dass die Diffusion spontan erfolgt mit einer der jeweiligen Konzentration proportionalen Geschwindigkeit; deshalb führe man α als Zerfallskonstante des Salzes ein, so erhält man unter Beachtung der beiden Annahmen:

$$0 = D \cdot \frac{d^2\rho}{dx^2} - \alpha\rho \quad \dots\dots(10),$$

d.h. Konzentrationsverminderung durch spontanen Zerfall des Salzes und Konzentrationszunahme infolge der Diffusion gleichen einander aus.

Einen derartigen Zustand kann man sich z. Bsp. in folgender Weise realisiert denken. Am Boden der mit Zellsaft gefüllten Zelle sei eine dünne Haut des Salzes vorhanden, das mit konstanter Celerität zu diffundieren beginnt. Aus der Annahme, dass an der Aussenfläche der Flüssigkeit oder an der Zellmembran die Konzentration konstant bleibt, folgt, dass die den betr. Zustand definierenden Grössen von der Zeit unabhängig gesetzt werden können. So ergibt sich statt des partiellen der totale Differentialquotient:

$$\frac{d^2\rho}{dx^2} = \frac{\alpha}{D} \cdot \rho \quad \dots\dots(11).$$

Nunmehr mache man den Ansatz:

$$\rho = e^{ax} \quad \dots\dots(12),$$

und versuche, den Wert von a zu finden, der diese Gleichung zu einem Integral jener macht. Durch zweimaliges Differenzieren nach x ergibt sich:

$$\begin{aligned} \frac{d\rho}{dx} &= ae^{ax}, \\ \frac{d^2\rho}{dx^2} &= a^2 e^{ax} \quad \dots\dots(13). \end{aligned}$$

In Gleichung (11) ist zu substituieren: ρ durch den Ausdruck (12) und $\frac{d^2\rho}{dx^2}$ durch den zuletzt gefundenen Wert (13), so resultiert:

$$a^2 e^{ax} = \left(\frac{\alpha}{D}\right)^2 \cdot e^{ax}.$$

Zur Vereinfachung setze man $\frac{a}{D} = m$ und kürze beide Seiten der Gleichung durch e^{ax} :

folglich $a^2 = \pm m$,
mithin sind $\phi_1(x) = e^{mx}$ und $\phi_2(x) = e^{-mx}$ zwei partikuläre Integrale der Gleichung (11) in der abgeänderten Form $\frac{d^2\rho}{dx^2} = \left(\frac{a}{D}\right)^2 \cdot \rho$. Das allgemeine Integral würde sich also linear zusammensetzen:

$$\rho = C_1 e^{mx} + C_2 e^{-mx} \quad \dots (14).$$

Bei der Bestimmung der beiden Integrationskonstanten C_1 und C_2 sei vorausgesetzt, dass die Konzentration an der Aussenfläche = 0 gesetzt werden kann. Ist h die Höhe der Zelle, so erhält man:

$$\begin{aligned} a &= C_1 + C_2, \\ 0 &= C_1 e^{mh} + C_2 e^{-mh}, \end{aligned}$$

und daraus leicht die Gleichungen:

$$\begin{aligned} C_1 &= \frac{ae^{-mh}}{e^{mh} - e^{-mh}}, \\ C_2 &= \frac{ae^{-mh}}{e^{mh} - e^{-mh}}, \end{aligned}$$

deren Werte in die Gleichung (14) substituiert werden müssen:

$$\rho = \frac{a}{e^{mh} - e^{-mh}} (e^{m(h-x)} - e^{-m(h-x)}) \quad \dots (15),$$

d.h. bei stationärem Zustande ist das Salz in der Zellflüssigkeit verteilt.

Man kann auch die Gesamtmenge der in der Zelle enthaltenen Salzsubstanz errechnen. Nach einem bekannten Satze der Integralrechnung¹ erhält man:

$$\begin{aligned} \int_0^h \rho(x) dx &= \int_0^h \frac{a}{e^{mh} - e^{-mh}} (e^{m(h-x)} - e^{-m(h-x)}) dx \\ &= \frac{a}{e^{mh} - e^{-mh}} \left[e^{mh} \int_0^h e^{-mx} dx - e^{-mh} \int_0^h e^{mx} dx \right] \\ &= \frac{a}{e^{mh} - e^{-mh}} \left[e^{mh} \left(-\frac{1}{m} \cdot e^{-mx} \right)_0^h - e^{-mh} \left(\frac{1}{m} \cdot e^{mx} \right)_0^h \right] \\ &= \frac{a}{m} \cdot \frac{1}{e^{mh} - e^{-mh}} [e^{mh} (1 - e^{-mh}) - e^{-mh} (e^{mh} - 1)] \\ &= \frac{a}{m} \cdot \frac{1}{e^{mh} - e^{-mh}} (e^{mh} - 2 + e^{-mh}). \end{aligned}$$

¹ Ist die Konzentration ρ einer Substanz in einem Zylinder als Funktion der Höhe x gegeben, also $\rho = \rho(x)$, so ist die zwischen den Höhen a und b enthaltene Menge der Substanz $= \int_a^b \rho(x) dx$.

Da nun für $\eta \rightarrow \infty$ die Gesamtquantität der Diffusion gegen $\frac{a}{m}$ konvergierend anzuspochen ist, indem

$$\lim_{\eta \rightarrow \infty} \frac{e^{m\eta} - 2 + e^{-m\eta}}{e^{m\eta} - e^{-m\eta}} = \lim_{\eta \rightarrow \infty} \frac{1 - \frac{2e^{-m\eta}}{e^{m\eta} - e^{-m\eta}} + \frac{e^{-2m\eta}}{e^{m\eta} - e^{-m\eta}}}{1 - \frac{e^{-2m\eta}}{e^{m\eta} - e^{-m\eta}}} = 1$$

ist, so ergibt sich:

$$\int_0^\infty \rho(x) dx = \frac{a}{m} \quad \dots (16).$$

Experimentell wurde die Frage, wieviel Oxalat zu Ca-haltiger Lösung gebracht werden kann, damit die Löslichkeitsgrenze des entstehenden Calciumoxalats soeben überschritten wird, durch R. Brinkman und Miss van Dam untersucht. Sie fanden das "Löslichkeitsprodukt" $[Ca^{++}] \cdot [C_2O_4^{--}]$ konstant zu 0,555 Millimole im Liter bei 20° C. Da nun ausserdem neben der $[H^+]$ die Menge der in Lösung befindlichen Bioxalationen bestimmend für die Löslichkeit oder Auskristallisierung des Calciumoxalats gefunden wurde, wird sich die Darlegung der Resultate selbst angestellter Experimente im Rahmen der vorliegenden Untersuchungen über den Einfluss der $[H^+]$ erübrigen.

2. Über die Relation der $[H^+]$ zur Morphologie der $Ca \cdot C_2O_4$ -Kristalle.

Schon J. Möller (p. 433) fiel auf, dass in sekundären Rinden Kristalldrüsen, Sand und Raphiden fast ausnahmslos in dünnwandigen, wohl ausgebildete Einzelkristalle dagegen vorwiegend in sklerotischen Zellen oder in der unmittelbaren Nachbarschaft solcher vorkommen. Der Autor möchte diese Erscheinung auf eine Beschleunigung resp. Verzögerung der osmotischen Prozesse zurückführen. Durch verschiedene Beobachtungen wird diese Anschauung scheinbar gestützt. So werden in der primären Rinde junger Internodien, die durch lebhaften Stoffumsatz charakterisiert sind, meistens Drüsen angetroffen. Mit der Anlage des Periderma als einem die Transpiration verzögernden Mantel überwiegt dann durchschnittlich die Bildung von Einzelkristallen. Auch in älteren Internodien und besonders in den sklerotisierten Partien der sekundären Rinde der Pflanzen scheinen die Drüsen fast durchgängig in Einzelkristalle umgebildet zu sein. In diesem Sinne würde auch der Wechsel der Kristallform bei manchen *Corylaceen*, *Thymelaeaceen*, *Rutaceen*, *Simarubaceen*, *Malpighiaceen*, *Ampelopsidaceen*, *Sapotaceen*, *Caprifoliaceen* usw. zu verstehen sein, bei denen die Kristallzellen der sekundären Rinde später von sklerotisierten Elementen umwachsen werden und nun erst die Umkristallisierung der anfangs gebildeten Drüsen erfolgt. Ebenso könnte das Vorkommen von Einzelkristallen in den verdick-

ten Elementen der Kammerfasern in dem angegebenen Sinne eine zureichende Deutung erfahren. Wenn aber gegenüber den angeführten Argumenten auch in jüngsten Internodien und in völlig sklerenchymfreien sekundären Rinden Einzelkristalle auftreten, so müssen wir folgern, dass mit der verschiedenen Wanddicke nur einer der Faktoren erkannt ist, die zur Verzögerung resp. Beschleunigung der Diosmose und damit zu einem bestimmten Kristallisationsmodus führen. So sieht auch Haberlandt (1904, p. 467; 1918, p. 492) einen weit erheblicheren Einfluss in der grösseren oder geringeren *Stoffwechselenergie der Gewebe* im allgemeinen, und neben derartigen ernährungsphysiologischen Faktoren hält er die *spezifische Konstitution des Plasmas* in den betr. Kristallbehältern für gleich bedeutsam bei der Ausbildung der verschiedenen Kristallformen des Calciumoxalats. Die Einsicht in die Mechanik der letztgenannten Gewebegeschehen sei (*l.c.*) unmöglich¹.

Der Umstand nun, dass eigene Untersuchungen die Ausbildung von Trennungsmeristemem zum Abwurf von Blättern (Pfeiffer, 1924) ernährungsphysiologisch auf die Anstauung von Nährsubstanzen (Kohlehydraten)² zurückführten, währenddessen durch Priestley, Ewing, Herklots u.a. die Auslösung von Zellteilungen durch *Variation der [H⁺]* angenommen wurde, liess die Vermutung entstehen, dass die anfangs angenommenen und in der Literatur mehrfach bestätigten ernährungsphysiologischen Beziehungen bei der Bildung verschiedener Kristallisationsformen des Calciumoxalats in der sekundären Rinde der Pflanzen vielleicht ebenso nur eine Umschreibung der $[H^+]$ und $[OH^-]$ einschliessen könnten. Es war dann anzunehmen, dass die ungleiche Grösse der Drusen und Einzelkristalle, ihr zunehmender Abstand in manchen Rindenpartien, die Umkristallisierung der drusigen Kristallaggregate u.v.a. aus Phänomenen der Konzentrationsgefälle resultieren, von denen nunmehr versucht werden sollte, sie wenigstens teilweise aufzudecken. Entsprechend dieser gewandelten Ansicht über das faktische Wesen der Acidität

¹ Es muss zugegeben werden, dass die "Conformationsen" bewirkenden inneren Komplementärbedingungen der Gewebegeschehen vorläufig sowohl nach ihrem Ursprung, als auch nach der Art ihrer Wirksamkeit unbekannt sind (Pfeiffer, 1925 b), und die genaue Erforschung der äusseren Komplementärbedingungen, durch die die "Gewebemorphosen" hervorgerufen werden, mag auch eine besonders wichtige Aufgabe der vorerst zu lösenden künftigen Fragen darstellen. Dennoch brauchen wir nicht unbedingt so pessimistisch zu urteilen, die Wirkung innerer Bedingungsweisen uns *a priori* verschlossen zu halten; man vgl. nur diesbezüglich die klaren Gedankengänge Küsters (1923) über Gewebekorrelationen. Unsere Untersuchungen über die Einwirkung der $[H^+]$ brauchen also nicht unbedingt zum Scheitern verurteilt zu sein.

² Vgl. auch den II. Hauptabschnitt dieser Abhandlung.

sollte also weder auf unauffindbare Säuren, noch auf kolloidale Besonderheiten des Zellplasmas zurückgegriffen, sondern jede Lösung allein nach ihrem Gehalt an freien H-Ionen beurteilt werden.—Es sei vorweg genommen, dass es bislang nur erst gelungen ist, gewisse Beziehungen der $[H^+]$ zum Kristallisationsmodus des Calciumoxalats aufzudecken.

Mit den Bedingungen, die die Ausbildung der Calciumoxalatkristalle beeinflussen, haben sich in älterer Zeit namentlich Vesque, Kny und Kohl beschäftigt. Schon vorher teilten Souhay und Lenssen mit, dass bei rascher Bildung das Oxalat mit 2 Äquivalenten Kristallwasser (monoclin), bei langsamer Ausscheidung solches mit 6 Molekülen H_2O (tetragonal) entsteht; allerdings gaben sie zu, dass beide Kristallformen aus derselben Mutterlauge entstehen können¹. Nach Haushofer sollte das tetragonale Salz aus verdünnten, ammoniakhaltigen, neutralen oder alkalischen Lösungen bei gewöhnlicher Temperatur, das monocline dagegen bei Gegenwart freier Salzsäure und überschüssiger Oxalsäure aus kochend heissen Lösungen entstehen. Kny gelangte zu der Überzeugung, dass weder die saure, noch die alkalische Reaktion der Mutterlauge von wesentlichem Einfluss auf den Wassergehalt der Kristalle und damit auf die Art des Kristallsystems sei, dass dagegen der relative Konzentrationsgrad der beiden Lösungen, durch deren Zusammentreffen die Bildung der Calciumoxalatkristalle bedingt sei, wenn nicht ausschliesslich, so doch von erheblicher Bedeutung sei. Sehr viel umfassender sind die Versuche von Kohl, deren Resultate sich mit den eigenen über diesen Gegenstand mehr oder minder decken, nur dass diejenigen jenes Autors den Einfluss der $[H^+]$ nicht untersuchen.

Anorganische Experimente.

Man lässt auf gelatinierten oder mit Eiweiss überzogenen Objektträgern ausser einer Oxalsäurelösung eine solche eines Calciumsalzes langsam zueinander hin diffundieren. Event. kann man die Lösung

¹ Eine für unsere Zwecke ausreichende Anleitung für die Ermittlung des Kristallsystems findet sich bei Fuchs-Braun, *Anleitung zum Bestimmen der Mineralien*, Giessen, 1907, p. 71. Wir können danach unterscheiden:

(a) Die meisten Kristalle werden zwischen gekreuzten Nicols hell (oder grau) und farbig und besitzen eine gerade Auslöschung, d. h. die Richtung, in der sich ein Kristall wie ein einfach-brechender verhält, läuft der Hauptkante parallel (Bestimmung durch den Teilkreis am Analysator). Einzelne Kristalle bleiben in allen Lagen dunkel, aber alle sind doppelbrechend und optisch einachsigt: *tetragonale Kristalle*;

(b) Alle Kristalle werden zwischen gekreuzten Nicols hell (oft nur grau) und farbig und sind optisch zweiachsigt. Die meisten besitzen eine schiefe Auslöschungsrichtung, die mit der Hauptkante des Kristalls einen Winkel bildet: *monocline Kristalle*.

des Salzes vermittelt eines Fliesspapierstreifens langsam der Säurelösung zuleiten. Man erhält so die auch in der natürlichen Umgebung beobachteten Kristallformen des entstehenden Calciumoxalats. Um die Beziehung der $[H^+]$ zu den beobachteten Kristallformen aufzudecken, bedarf es nur der Anwendung von Indikatoren, deren Veränderung mit genau definierbaren Kontrolllösungen auf weisser Milchglasscheibe leicht verglichen werden kann. Wegen der Schwierigkeit der Durchdringung des ganzen Problemkomplexes sind vorläufig nur erst die folgenden 10 Indikatoren zu den Untersuchungen herangezogen worden¹.

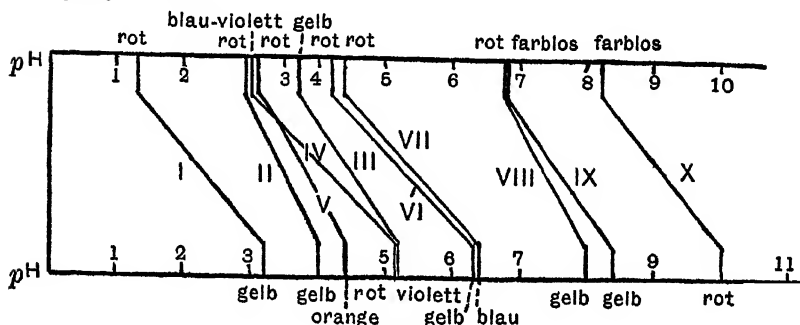


Fig. 1.

Nr.	Name	chemische Zusammensetzung	pH
I.	Tropäolin	Diphenylaminoazo- <i>p</i> -benzolsulfonsäurenatrium $\text{SO}_3\text{NaC}_6\text{H}_4\text{N}=\text{NC}_6\text{H}_4\text{NHC}_6\text{H}_5$	1,3–3,2
II.	Dimethylgelb, butter-yellow	Dimethylaminoazobenzol $\text{C}_6\text{H}_5\text{N}=\text{NC}_6\text{H}_4\text{N}(\text{CH}_3)_2$	2,9–4,0
III.	Alizarinsulfon- säurenatrium	$\text{C}_6\text{H}_3\langle\text{CO}\rangle\text{C}_6\text{H}(\text{OH})_3\text{SO}_3\text{Na}$	3,7–5,2
IV.	Congorot	Natriumsalz der Benzidindisazo- <i>m</i> -amidobenzol- sulfosäure-1-naphthylamin-4-sulfosäure $\text{SO}_3\text{Na}\rangle\text{C}_{10}\text{H}_8\text{N}=\text{NC}_6\text{H}_4\text{C}_6\text{H}_4\text{N}=\text{NC}_{10}\text{H}_6\langle\text{SO}_3\text{Na}\rangle\text{NH}_2$	3,0–5,2
V.	Methylorange	Dimethylaminoazobenzolsulfonsäurenatrium $\text{SO}_3\text{NaC}_6\text{H}_4\text{N}=\text{NC}_6\text{H}_4\text{N}(\text{CH}_3)_2$	3,1–4,4
VI.	Methylrot	Dimethylaminoazobenzol- <i>o</i> -carbonsäure $\text{COOH}\text{C}_6\text{H}_4-\text{N}=\text{NC}_6\text{H}_4\text{N}(\text{CH}_3)_2$	4,2–6,3
VII.	Lakmoid	$\text{C}_{13}\text{H}_9\text{O}_3\text{N}$	4,4–6,4
VIII.	Neutralrot	α -dimethyldiaminophenazinchlorid $\text{N}(\text{CH}_3)_2\text{C}_6\text{H}_3\langle\text{N}\rangle\text{C}_6\text{H}_4\text{CH}_2\text{N}(\text{CH}_3)_2$	6,8–8,0
IX.	<i>m</i> -Nitrophenol	$\text{C}_6\text{H}_4\text{NO}_2\text{OH}$	6,8–8,4
X.	Phenolphthalein	$\text{C}_6\text{H}_3\langle\text{C}\rangle\text{O}$ $[\text{C}_6\text{H}_4\text{OH}]_2$	8,2–10

¹ Vgl. zu der Eigenart dieser und anderer Indikatoren etwa: Sörensen (p. 161 sq.), G. Schultz und Julius Treadwell (p. 462 sq., mit weiteren Literaturangaben), sowie Kolthoff (p. 44 sq.); der letztere zitiert: E. B. R. Prideaux, *The use and application of indicators*, 1917.—Vgl. auch Fig. 1.

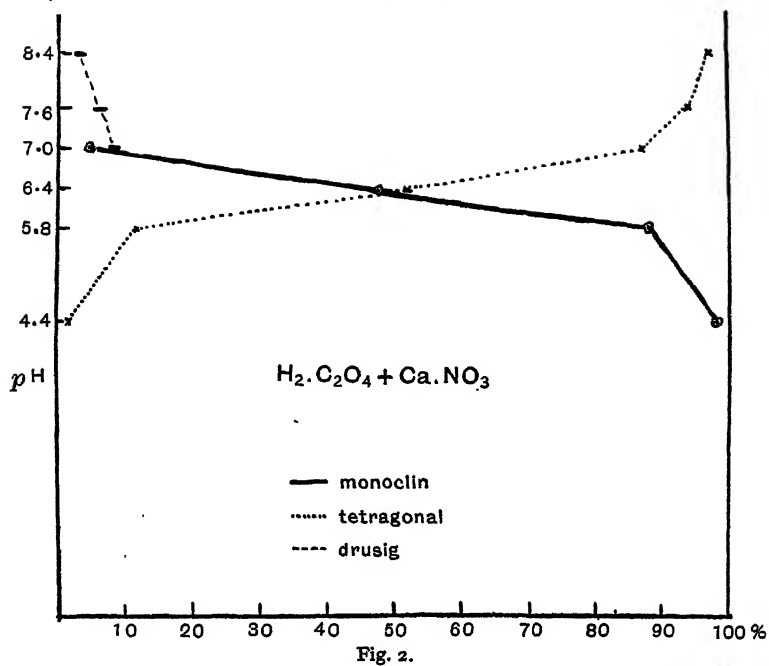


Fig. 2.

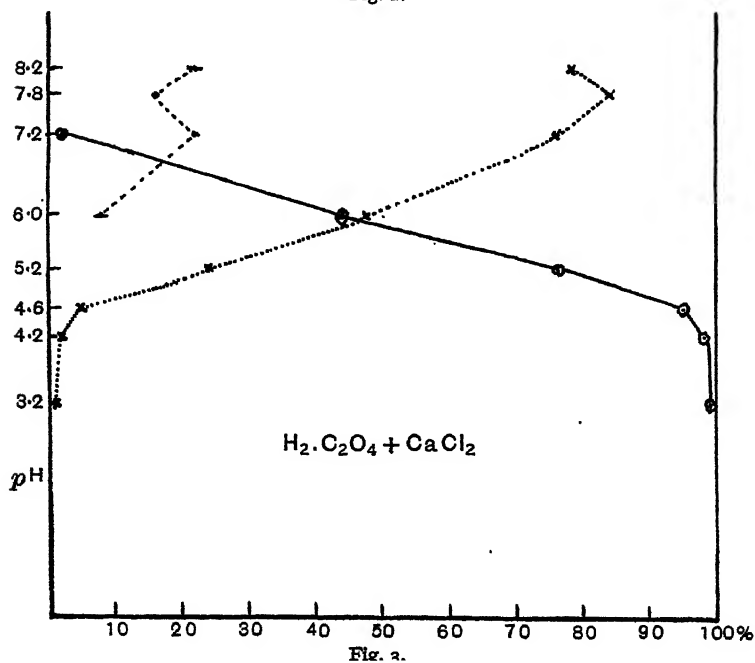
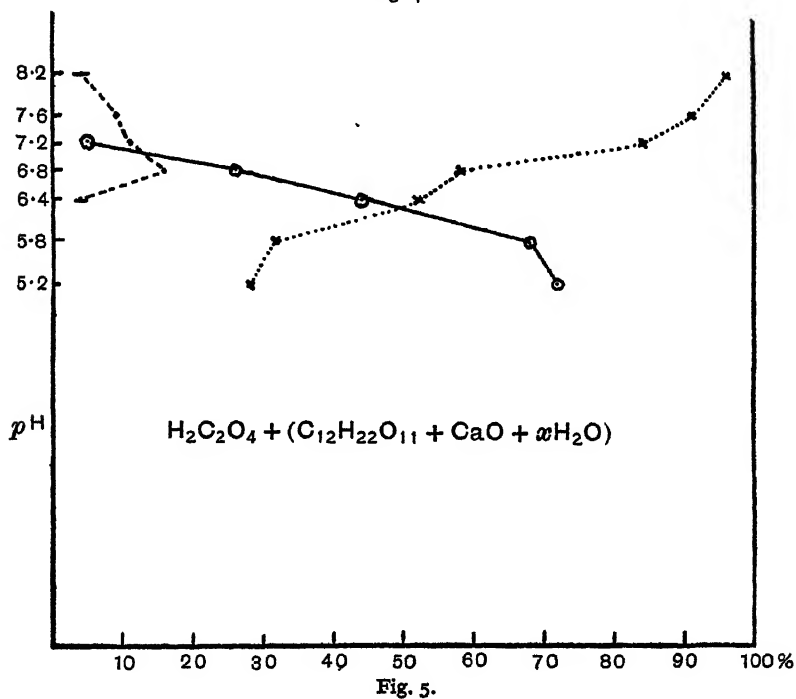
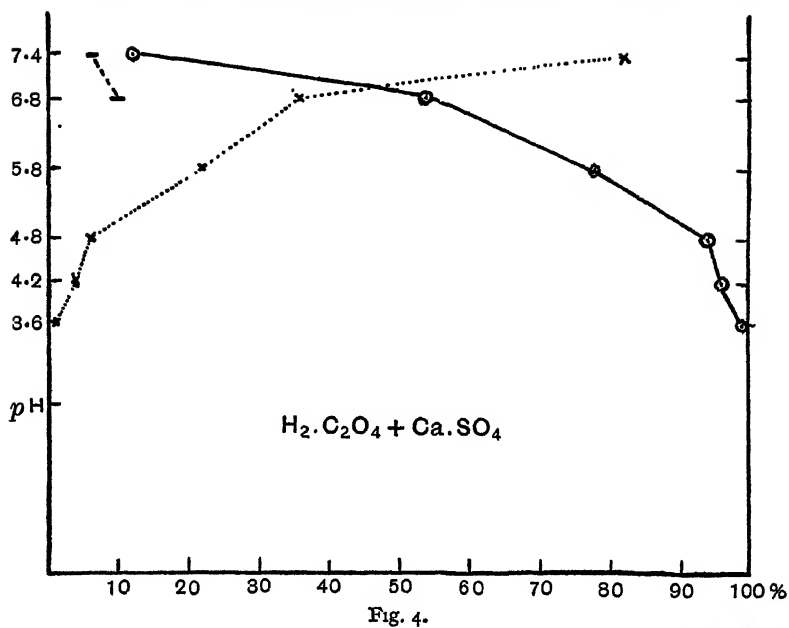


Fig. 2.



Bemerkungen zu den angestellten Experimenten.

Die Beobachtung von Kny, dass die Oxalsäure langsamer diffundiert als die mit ihr kombinierten Calciumsalze, ist zu bestätigen. Es zeigt sich also, dass die Kristallisationszone derjenigen Seite des Objektträgers, der man die Oxalsäure aufgetragen hat, näher liegt als der andern. Beim Vergleich der angestellten Experimente machen sich je nach der chemischen Zusammensetzung der Calciumsalze gewisse Variationen in der Abhängigkeit der Kristallform von der $[H^+]$ bemerkbar. Im allgemeinen fällt aber auf, wie die wasserärmere, *monocline Kristallform für saure pH-Werte charakteristisch* ist, obzwar auch schon bei $pH < 7,0$ tetragonale Kristalle des Calciumoxalats auftreten können. Doch ist diese wasserreichere Kristallform häufiger bei neutralen und basischen pH-Werten, sofern wir den isoelektrischen Punkt zwischen $pH\ 6,0-7,0$ annehmen. Ergänzend sei erwähnt, dass bei gleichzeitiger Bildung monocliner und tetragoaler Kristalle entgegen Kny und in Übereinstimmung mit Kohl zuerst die für saure Reaktionsweise charakteristischen monoclinen und erst dann tetragonale Kristallformen aufzutreten pflegen. Besonders die tetragonalen Kristalle wachsen in der Regel längere Zeit fort und erreichen dabei auch stattlichere Dimensionen. Diejenigen in sekundären Rinden auftretenden Einzelkristalle, die allein oder annähernd eine ganze Zelle erfüllen, dürften somit nicht allein nach ihrem optischen Verhalten, sondern auch entsprechend den angestellten Experimenten in der Regel tetragonale Formen darstellen.

Erst bei alkalischen pH-Werten kommt es in den Experimenten zur Bildung drusiger und sphärischer Kristallaggregate—die beiden Modi werden hier zusammengefasst. Sie resultieren wohl meistens aus Kristallen von der Form $\propto P \cdot oP$ oder $\propto P \cdot oP \cdot mP \propto$, gehen aber bisweilen auch auf Kristalle der Formel $\propto P \cdot oP \cdot \infty P \propto$ oder Zwillingsbildungen $\propto P \propto \cdot \infty P \propto$ — P zurück. Freilich gelingt es nicht immer, die Drusen theoretisch in ihre aufbauenden Einzelkristalle zu analysieren. Der für pflanzliche Zellen charakteristische Zentralkörper (*Sanio*) oder *corpo mucilaginoso* (*Buscalioni*) in den Drusen scheint nach den bisherigen Resultaten immer (oder häufig?) den auf den Objektträgern experimentell erzeugten Aggregaten zu mangeln.

Temperaturunterschiede haben, soweit die Temperaturvariationen das Niveau der für die Pflanzen in der natürlichen Umgebung in Betracht kommenden nicht wesentlich überschreiten, keinen Einfluss auf die Kristallisationsbedingungen des Calciumoxalats im allge-

meinen und auf die Entstehung der verschiedenen Kristallformen im besonderen. Die Mitteilung entsprechender Versuchsreihen, die zur Aufklärung dieser Frage ausgeführt wurden, darf somit wohl übergangen werden.

Zusammenfassend kann vorerst konstatiert werden, dass sich längst vergessene Anschauungen Haushofers über die Bedingungen der Kristallisation des Calciumoxalats bestätigt haben; freilich müssen seine Ansichten in beschränktem Masse auch verworfen werden.

Anatomisch-indikatorische Beobachtungen an sekundären Rinden.

Nachdem die Scrien anorganischer Experimente gezeigt haben, dass die Kristallformen des Calciumoxalats grossenteils durch den pH -Wert der Kristallisationslösung determiniert werden, wird es nötig, durch entsprechende Versuche an lebenden Rinden die angeführte Folgerung noch mehr zu erhärten. Während nun die meisten in Anwendung befindlichen Untersuchungsverfahren den Nachteil aufweisen, dass beim Vergleich benachbarter Gewebepartien eines Querschnittes vorkommende Differenzen nicht oder nur schwierig zu konstatieren sind, ist durch Atkins die Methode gegeben, die unseren Anforderungen genügt. Er behandelt Querschnitte durch die zu untersuchenden Pflanzenorgane mit verschiedenen Indikatorlösungen, deren pH für die wechselnden Farbtöne genau festgelegt ist. So wird es möglich, auf dem Querschnitt die Acidität der einzelnen Gewebzone sofort abzulesen.—Die Untersuchung von Presssäften birgt dem gegenüber sicher eine Reihe von Fehlerquellen; nicht zum wenigsten liegt die Ungenauigkeit dieser Methode auch darin, dass der Zellsaft mit den in ihm gelösten Stoffen mit den Plasma- und Zellwandteilchen in Reaktion treten muss. Weiterhin werden auch Reaktionen zwischen den Ampholyten des Protoplasten und den im Zellsaftraum befindlich gewesenen Säuren resp. sauren Salzen eintreten müssen (vgl. Mevius, p. 657, ferner Atkins, u.a.).

Nach Arrhenius haben wir die pflanzlichen Zellen als ein System teils dissoziierter, teils undissoziierter Ampholyten in Mischung mit Wasser, Luft und geringen Salzquantitäten aufzufassen. Die Tatsache sukzessiver Variation der Acidität ergibt bei der Durchführung der Beobachtungen an lebenden Sekundärrinden zwar sehr interessante Einzelresultate, erschwert aber die Möglichkeit, die gefundenen Werte in einer Regel zusammenzufassen. Nach Atkins reagieren die Pflanzenzellen selten alkalisch ($pH = 8,0$ wird nicht überschritten), während

andererseits Werte von $pH = 1,4$ häufig realisiert sind. Hoagland und Davis (1922) fanden die $[H^+]$ lebenskräftiger Zellen nahezu konstant einem pH -Werte von 5,2; dieser liess sich auch nicht beeinflussen, wenn der pH -Wert des Aussenmediums von 5,0 bis 9,0 variiert wurde. Ebenso ist die Reaktion in pflanzlichen Zellen zufolge Haas mehr oder minder sauer, und die $[H^+]$ sinkt erst beim Absterben der Zellen von $pH = 3,0$ auf $pH = 7,0$. Nach diesen Autoren sind somit, wenn auch nur in geringem Grade, in lebenden Pflanzenzellen die Relationen annähernd gegeben, die wir nach unsern anorganischen Experimenten für die Bildung von Calciumoxalatkristallen gefolgert haben. Wir müssen aber weiterhin bedenken, dass die Ansicht von der alkalischen Natur der Pflanzenzelle bez. von der Gegenwart eines Überschusses an OH -Ionen in ihnen sich weit allgemeinerer Beliebtheit erfreut. So sollen sich die Zellen nach Lepeschkin (1924, p. 109) meistens alkalischer als $pH = 7,0$, freilich bisweilen auch sicher neutral oder sauer verhalten. Ähnlich tritt auch Tunmann (p. 441) für die alkalische Reaktion des Plasmas der organisierten Bestandteile der Zellen ein und nimmt nur für den Zellsaft der Pflanzen saure Reaktion an. Wenn auch die Methodik von Schaepe,—Verwendung basischer Vitalfarbstoffe—wie Ruhland (1923, p. 253) gezeigt hat, zu Beanstandungen Anlass geben kann, so wird von dem letztgenannten Kritiker an der Annahme der alkalischen Reaktion der Pflanzenzellen kein Zweifel ausgesprochen. Auch nach Lundegårdh (1922, p. 202) findet sich ein Überschuss freier H -Ionen nur in dem deutlich sauer reagierenden, eiweissarmen Zellsaft, während das Cytoplasma schwach alkalisch reagiert. Er hält es freilich für nicht sichergestellt, wieweit solcher negativen Ladung des Eiweisses eine eigene Ionisation zugeschrieben werden muss oder aus der Aufladung der Elektrolyte resultiert (vgl. Höber, p. 321, 330, 475 u.a.). Der durch wochenlange Dialyse möglichst von anhaftenden Elektrolyten befreite Eiweissampholyt ist nach Pauli stärker anodisch als kathodisch, d.h. die Säuredissoziationskonstante überwiegt ein wenig die der Basen. Nach Stoklasa (p. 184) ist auch der Zellsaft nicht so sauer, wie bislang angenommen wurde, bisweilen sogar fast neutral. Ein Ansteigen der $[H^+]$ ist nach ihm vor allem durch den Mangel an O hervorgerufen, wodurch bei Leguminosen beispielsweise ein pH -Wert von 5,0 bis 6,0 resultieren kann. Sollten sich also die Angaben der zuletzt zitierten Autoren uneingeschränkt bestätigen, so wäre unser Versuch einer kausalen Erkenntnis der Kristallisationsphänomene des Calciumoxalats fast fehlgeschlagen. So viel scheint aber im voraus festzustehen, dass in den dispersen Phasen, die pflanzliche

Eiweisskörper enthalten, und vielleicht in der ebenfalls eiweisshaltigen Grundmasse des Protoplasmas nicht oder nur schwer diffundierende Eiweissionen (bei saurer Reaktion Kationen, bei alkalischer natürlich Anionen) auftreten. Wir dürfen daher mit Donnan zwischen dem Protoplasma s. str. und dem umgebenden elektrolythaltigen Wasser die Existenz eines elektrischen Potentials postulieren, so dass das Plasma negativ geladen wird (vgl. J. Loeb, p. 151–56). Durch Zusatz kleiner Quantitäten von Säuren oder Basen muss sich die "gewöhnlich negative" Ladung (?) in manchen Fällen modifizieren lassen, weil wahrscheinlich saure oder alkalische Reagentien in die Zelle eindringen und mit Kolloiden des Protoplasmas entsprechende Verbindungen eingehen können (vgl. Höber, p. 300), so dass nach innen nicht-diffundierende Kationen oder Anionen entstehen (s. Stern, p. 25 und die dort angegebene Literatur!).

Experiment 5.

Die vorstehende gedankliche Durchdringung der einschlägigen Fragen ergibt annähernd einen *Einblick in die zu überwältigenden Schwierigkeiten*, die der Erprobung der erkannten Grundsätze an Präparaten lebender Rindengewebe der Pflanzen entgegenstehen. Sie werden noch erheblich dadurch vergrössert, dass die Calcium-oxalatkristalle teilweise im Laufe eines Tages wachsen oder sich vermindern können (Alexandrov und Prichodjko). Um nun innerhalb der Grenzen des Möglichen alle Verhältnisse entsprechend denen in der natürlichen Umgebung pflanzlicher Rindenzellen zu erfüllen, wird von besonderen Düngungs- und Ernährungsversuchen der untersuchten Pflanzen abgesehen. Vielmehr gelangen allein Querschnittserien von der sekundären Rinde einer ganzen Reihe von Laubbölzern Mitteleuropas in entsprechenden Indikatorenlösungen als Untersuchungsmedium zur Beobachtung. Bislang sind unter den weiter oben aufgeführten Indikatoren bei der mikroskopischen Untersuchung die folgenden fünf bevorzugt worden: (1) Tropäolin 00, (2) Dimethylgelb, (6) Methylrot (vgl. E. Rupp und R. Loose in *Ber. Deutsch. Chem. Ges.* 1908, 41, p. 3905!), (8) Neutralrot, (10) Phenolphthalein.

Da die Reaktionsgeschwindigkeit in heterogenen Systemen nach Annahme der Kolloidchemiker (vgl. Handovsky, p. 197) im wesentlichen von der Diffusionsgeschwindigkeit abhängt¹, ist versucht

¹ Vgl. Nernst und Brunner in *Zeitschr. f. physik. Chemie*, 1904, 47, p. 52! Als Beweis dafür werden angeführt, dass die Reaktionsgeschwindigkeit von der mechanischen Rührung abhängt, dass ihr Temperaturkoeffizient dem

worden, die Schnitte ohne Deckglas auf dem Objektträger zu untersuchen und erst dann ein solches zu benutzen. Der besseren Kontrolle wegen werden sämtliche Präparate nur bei künstlichem, am leichtesten möglichst konstant zu haltendem Lichte untersucht. Selbst die Blendenöffnung dürfte nach praktischen Erfahrungen für die ganz genaue Ermittlung des Farbgrades nicht gleichgültig sein. Auch wenn dieselbe Pflanze, ja dasselbe Individuum und sogar benachbarte Rindenkomplexe zur Untersuchung kommen, ist es nach den bisherigen Ergebnissen überaus schwer, unter den äusserst wandelbaren Verhältnissen die Regel zu erkennen. Besonders wichtig erscheint da die Untersuchung solcher Partien in den Rindengeweben, in denen mit Wahrscheinlichkeit jüngst die Bildung von Calciumoxalatkrystallen begonnen hat. Im besonderen muss daran festgehalten werden, dass die *Acidität der Elemente der sekundären Rinde fortwährend geändert* wird. Auch Gustafson stellt bekanntlich Änderungen der $[H^+]$ mit der Reife (1922), bzw. mit dem Alter der Pflanzenteile fest (1924). Wenn Arrhenius konstatiert, dass die Pflanzen im Laufe ihrer Entwicklung ihr *Aciditätsoptimum verändern*, so gilt diese Regel uneingeschränkt auch für die sekundäre Rinde im speziellen. Auch bei dieser zeigt die Acidität je ein Maximum auf der sauren und alkalischen Seite des isoelektrischen Punktes; ersteres dürfte nach den bisherigen Beobachtungen bei $pH = 2,4-3,8$, letzteres bei $pH = 7,2-8,6$ liegen. Von den angegebenen Indikatoren zeigt sich zunächst die durch Tropäolin oo anzunehmende Acidität als mehr oder minder ungeeignet für jegliche Ausbildung von Calciumoxalatkrystallen. Dimethylaminoazobenzol und Methylrot stellen denjenigen Aciditätsgrad dar, bei dessen Realisierung nur (oder hauptsächlich?) monocline Kristalle zur Ausbildung gelangen. Der durch Neutralrot bestimmte Aciditätsgrad ist für die Bildung von tetragonalen und teilweise auch drusigen oder sphäritischen Kristallen massgebend, während endlich Phenolphthalein als für die betr. Untersuchungen ungeeignet erkannt wurde. Daraus wird gefolgert, dass bei—wenn auch nur vorübergehender—saurer Reaktion (bis $pH = 5,0$) durchgängig monocline, von $pH = 5,0-6,5$ in der Regel tetragonale, bei stark alkalischem pH -Werte neben tetragonalen drusige¹

der Diffusionsgeschwindigkeit entspricht, dass endlich Verzögerungen der Diffusionsgeschwindigkeit (Steigerung der Viscosität des Mediums) die Reaktionsgeschwindigkeit verkleinern. S auch die Darstellung bei Freundlich, p. 518 sq.

¹ Wie bereits bemerkt, zeichnen sich die Drusen lebender Rindengewebe gegenüber experimentell erzeugten durch die Existenz eines Centralkörpers aus. Dieser lost sich in Alkohol, Äther, Chloroform, und Schwefelsäure ziemlich rasch, in Chromsäure langsamer. Durch Iodalkohol wird er schwach

oder sphäritische Kristallfällungen auftreten. Sofern es sich um besonders weitleumige Gewebeelemente handelt, braucht nicht die ganze Zelle die entsprechende Reaktion zu zeigen; doch ist, von ganz vereinzelter, bislang noch nicht genügend erklärbarer Ausnahmen abgesehen, in unmittelbarer Nachbarschaft der entstehenden Calciumoxalatkristalle je nach deren spezieller kristallographischer Morphologie fast die nach unsern anorganischen Experimenten zu erwartende Reaktion verwirklicht.—Wenn die verschiedenen Kristallformen, von der Regel abweichend, bei einer andern $[H']$ auftreten, so könnte das übrigens in diesen Fällen—auch nach den anorganischen Versuchen—an der Art des die Metallionen abgebenden Salzes liegen. Es bedarf weiterer Ausdehnung der Versuche mit dem Ziel, zu prüfen, ob $CaCl_2$ das Optimum der Kristallisation ebenso nach der sauren Seite hin verschiebt, wie dies Lundegårdh (1924) für das Wachstumsoptimum von Gibberella gefunden hat.

Experimente 6 und 7.

Bereits Emich (p. 1138) benutzt zur Untersuchung der $[H']$ und $[OH']$ ein Verfahren, das—in abgeänderter Form—auch bei der Prüfung dieser Verhältnisse an sekundären Rinden der Pflanzen gute Dienste zu leisten verspricht. In derselben Weise, in der er Lakmusseide herzustellen und zu verwenden pflegt, kann auch eine "Neutralrotseide" verwendet werden. Die Seidenfäden werden mit diesem Indikator gefärbt (0,5 g. in 300 cm.³ gelöst, dann auf 500 cm.³ Wasser angefüllt, Färbung etwa 1½ Std. im erwärmten Bade) und dann in fließendes Wasser gebracht. Zur Prüfung von Gewebeeraktionen ist der Faden dann derart zu verwenden, dass man ihn auf den hergestellten Querschnitt bringt, mit einem Deckgläschen bedeckt und einige Zeit dort belässt. Sobald die Temperatur ansteigt, wird der Seidenfaden durch Verdampfung von capillar unter dem Deckglase befindlichen Lösungen der Zellen in der Färbung verändert. Indem eine Übersichtszeichnung des Präparates mit dem aufgelegten Faden hergestellt wird, wobei auf die feinsten Abstufungen in der Färbung des Seidenfadens geachtet werden muss, kann aus den Variationen der Farbe der Grad der Acidität in Näherungswerten abgelesen werden.

Die Methode ist freilich etwas umständlich, ihre Empfindlichkeit ist aber immerhin erstaunlich, wenn sie auch die des von Emich

gelblich färbt. Ausserdem ist seine Substanz, die *Sanio* als Protein anspricht, mit Alkannatinktur, Anilinblau, Methylenblau und Säurefuchsin färbbar; ihre Cu-Verbindungen sind nach Tunmann (1913, p. 139) grün gefärbt.

dargelegten Verfahrens noch bei weitem nicht erreicht. Die Verwendung von Objektträgern und Deckgläsern aus Quarz oder von Glasgegenständen, die mit neutralem Paraffin überzogen sind, vermag die Genauigkeit unserer Methode noch um einiges zu erhöhen.

Soweit es sich um trockene Rinden von Museumsmaterial handelt, muss die Untersuchung der $[H^+]$ anders erfolgen. Hof (p. 275) empfiehlt für solche Fälle zur Konstataction des Alkalitätsgrades Iodeosin. Er geht davon aus, dass das Kaliumsalz des Tetraiodfluoresceins die Eigenschaft hat, sich in Wasser leicht zu einer intensiv roten Flüssigkeit zu lösen, in Äther, Chloroform und Toluol dagegen unlöslich zu sein. Hingegen löst sich die freie Farbsäure des Iodeosins, die aus dem Salz durch Ansäuern einer Lösung ausfällt, in Wasser kaum, leicht indessen in organischen Lösungsmitteln. Wenn man somit mit ätherischen Lösungsmitteln ausschüttelt, löst sich die freie Farbsäure hierin zu einer gelben Flüssigkeit. Behandelt man Schnitte einer trockenen Sekundärrinde mit der ätherischen Lösung der freien Farbsäure, so werden die "alkalisch" reagierenden Stellen des Objektes augenblicklich intensiv rot. Der wissenschaftliche Wert der Methode besteht darin, dass sie ein exaktes Bild der topischen Verteilung alkalisch reagierender Komplexe innerhalb der Rinde liefert. Die Bemühungen, den Hofschens Indikator durch einen für die vorliegenden Untersuchungen geeigneteren—z. Bsp. Neutralrot (?), Methylrot, Dimethylgelb—zu ersetzen, sind bisher noch nicht zu empfehlenswerten Vorschlägen verdichtet.

Die hier zuletzt kurz dargelegten Untersuchungen führten zu dem zu erwartenden und durch die Indikatorenstudien (p. 78) bereits skizzierten Resultat einer Beziehung der Kristallisationsform des Calciumoxalats zur $[H^+]$.

ANHANG.

Über Bedingungen für die Grösse der Calciumoxalatkristalle und für ihre zonenartige Verteilung in der sekundären Rinde.

Küster (1913, p. 51) wagt meines Wissens als erster den Schluss, die ungleiche Grösse von Drusen und den zunehmenden Abstand zwischen ihnen in pflanzlichen Geweben auf *Diffusionsprozesse* und *Wirkungen von Konzentrationsgefällen* zurückzuführen, wie sie sich in ähnlicher Weise bei Agarversuchen einstellen. Es sei bemerkt, dass auch H. P. Möller (p. 172) und Wisselingh (p. 203, 238 u.a.) ähnlich die rhythmischen Fällungen in Geweben mit Liesegangschen Ringen und Zonen vergleichen wollen. Zur Untersuchung ent-

sprechender Niederschläge legt Lehmann (p. 502) ein Deckglas auf den Objektträger und bringt an die eine Seite desselben einen Tropfen der einen Flüssigkeit—so gross, dass sie sich eben bis in die Mitte des capillaren Raumes zieht—und auf die andere so viel der andern, dass sie die zuerst aufgetragene zu verdrängen sucht. Benutzt man übersättigte Lösungen von Salzen in einem Gemisch von Alkohol und Wasser [Alkoholzusatz in wässrigen Lösungen], so erreicht man verschieden langsames Niederschlagen. Je schneller ein Niederschlag hervorgebracht wird, desto zahlreicher sind die Kristallisationsstellen, desto kleiner werden aber auch die gebildeten Kristalle. Bei stark verminderter Geschwindigkeit der Fällung werden diese weniger zahlreich, aber robuster (vgl. auch Guignet!). Einige eigene Experimente mögen teilweise die Bedingungen klarlegen, die derartige Eigentümlichkeiten der ausfallenden Calciumoxalatkrystalle bestimmen.

Experiment 8.

Lässt man, wie Küster (*l.c.*), Silbernitrat durch einen kaliumbichromathaltigen Agar (0,25 %, Merck-Darmstadt) diffundieren, so fällt das Silberchromat diffus aus. Die Niederschlagspartikel liegen in den äusseren Partien des Diffusionsfeldes in geringen Abständen, sind indessen sehr klein; weiter nach aussen liegen grössere, die aber voneinander weiter entfernt sind.

Experiment 9.

Unter den zahlreichen Versuchen Liesegangs (1911, p. 328) zur Nachahmung von Phänomenen in Organismen sind einige, die er zum Studium sogen. Formkatalysatoren angestellt hat. Sie können wohl auf die uns hier interessierenden Fragen einiges Licht werfen. Unter Abänderung seines Versuches der Diffusion von Silberverbindungen gelangen wir zu der folgenden Versuchsanordnung:

Zuerst benötigen wir einer CaCl_2 -haltigen Gelatine. Zu deren Herstellung werfe man in verdünnte HCl (vol. 1 : 1) so lange Kreidestückchen, wie diese sich unter Brausen auflösen. Die filtrierte Lösung enthält CaCl_2 und wird eingedampft, bis sie eine gewisse zähe Konsistenz zeigt. Aus dieser erhält man beim Erkalten grosse, spiessige Kristalle von $\text{CaCl}_2 + 6\text{H}_2\text{O}$, die mit der Gelatine (2 g. Gelatine in 20 cm.³ warmem Wasser lösen, $\frac{1}{30}$ des Volumens CaCl_2 -Kristalle zusetzen) gekocht werden. Nach deren Erkalten ist sie in der beabsichtigten Weise vorbereitet. Bringt man auf die Gelatine einen Tropfen einer Na_2SO_4 -Lösung, so entsteht nach Ausbreitung

dieses Salzes durch Diffusion eine mehr oder minder homogene, kreisrunde Scheibe von $\text{CaSO}_4 + 2\text{H}_2\text{O}$. Sodann trägt man ein Körnchen Kleesalz $[\text{KHC}_2\text{O}_4 + \text{H}_2\text{C}_2\text{O}_4 + 2\text{H}_2\text{O}]$ auf. Nun bilden sich statt der homogenen Ablagerung von CaSO_4 mehr oder minder deutliche Zonen von dichtem $\text{Ca} \cdot \text{C}_2\text{O}_4$ -Niederschlag abwechselnd mit Calciumoxalatärmeren.—Vgl. auch die Darlegungen bei Liesegang (1924, p. 53 sq.); mit der Fällung und Lösung von Kalkverbindungen beschäftigt er sich dort besonders im XIV. Kapitel.

Es ist nicht unbedingt nötig, wie Liesegang die verschiedene Diffusibilität der entstehenden Salze für diese Phänomene verantwortlich zu machen. Nach den Resultaten der oben dargelegten Untersuchungen wäre ein Einfluss der $[\text{H}^+]$ zum mindesten hypothetisch zu postulieren. In welcher Weise sich die $[\text{H}^+]$ wirksam erweisen sollte, etwa infolge von Gefällen der $[\text{H}^+]$, wäre allerdings noch zu untersuchen. Darüber hinaus wäre aber auch der experimentelle Beweis für die Tatsache eines derartigen Einflusses noch zu fordern. *Leider ist bislang keine Versuchsmethodik bekannt geworden, um dadurch die hypothetische Annahme zu stützen.*

Dass übrigens *auch hochmolekulare Verbindungen* zu derselben oder ähnlicher Zonenbildung potenziert sind, ergeben die Experimente von Bechhold (p. 244), der z. Bsp. die Entstehung Liesegangscher Ringe zeigen konnte, wenn er Gelatine und Serum zusammen im Reagenzglasle erstarren liess und das Gel mit H_3PO_4 überschichtete. Ohne auf Einzelheiten weiter einzugehen, mag es weiteren Untersuchungen vorbehalten bleiben, entsprechend den Perspektiven, die sich hier eröffnen, durch geschickte Versuchsanstellung die Bedeutung der $[\text{H}^+]$ für das Eintreten derartiger Phänomene klarzulegen.

II. ÜBER DAS VERHÄLTNISS DER METAZYTISCHEN POTENZ DER ELEMENTE DER SEKUNDÄREN RINDE ZU IHRER AKTUELLEN ACIDITÄT.

Als *metazytische Potenzen* fassen wir gleich Nusbaum (1912) und Pfeiffer (1925 a, p. 56) in dieser Betrachtung die Metaplasien in pflanzlichen Sekundärrinden zusammen, bei denen die Entwicklung mit einer Variation des bisherigen Gewebecharakters unter Ausschluss von Veränderungen durch Wachstum und Teilung von Elementen zu charakterisieren ist. Es wurde von Pfeiffer (*l.c.* p. 57) bereits darauf hingewiesen, dass nicht jede Membranproduktion unvergrössert und ungeteilt bleibender Rindenzellen metazytischer Natur ist. Wenn aber auch eine scharfe begriffliche Scheidung zwischen metazytischer und degenerativer Membransklerotisierung nicht leicht

zu formulieren ist, so wird doch *in praxi* meistens die Entscheidung weit weniger Schwierigkeiten bereiten.

Die *Zellmembranen* werden wir uns mit Hansteen-Cranner (1919, p. 387; 1922, p. 146) als ein kolloidales Netzwerk vorstellen, dessen festes Gerüst aus Cellulose und Hemicellulosen gebildet ist, dessen Maschen sodann Lipide der cytoplasmatischen Grenzschichten enthalten. Dass es unter den Lipiden auch zuckerführende gibt, muss für die Bildung und Verdickung der Zellmembranen dadurch bedeutungsvoll werden, dass jene direkt am Verwendungsorte als Baumaterial für letztere abgegeben werden können: beim Flächenwachstum der Häute durch Intussusception von den in ihnen selbst enthaltenen Lipiden, beim Dickenwachstum der Membranen durch Apposition der in den cytoplasmatischen Grenzschichten niedergelegten Zuckermoleküle der Lipide (*l.c.* p. 390; vgl. auch Pfeiffer, 1924).—Nach Wislicenus und Kleinstück (p. 87; s. ferner Wisselingh, 1925, p. 91 *sq.*) ist das Holz, abgesehen von der ursprünglichen vitalen Celluloseerzeugung im Plasma, als das Resultat vorwiegend kolloid-chemischer Prozesse, die sich aus Gelierung und Kolloidadsorption kombinieren, zu betrachten. In der ersten Stufe soll die zur Mizellen-, Gewebs- und Faserstruktur führende Cellulosegelbildung den chemisch indifferenten, unlöslichen Oberflächenkörper (das Adsorbens cellulose) im voraus bilden. Erst daraufhin dürfte in einem weiteren Stadium das Cellulosegel oder seine Quellungsprodukte von den kolloiden Saftstoffen, die im cambialen Saft der Pflanzen vermutlich durch Kondensations- und andere organisch-chemische Synthesen und durch kolloides Wachstum der Molekularkomplexe oder Amikronen zu Kolloidteilchen dauernd gebildet werden, teils durch Adsorption, teils durch Gelhautaufflagerung umhüllt werden.—Nur wenig modifiziert von der Anschauung Hansteen-Cranners scheint mir die Ansicht MacDougals. Dieser hält die pflanzliche Membran für ein maschiges Skelett von Cellulosefasern, dessen Interstitien im äusseren Teile von Pectinen und Pectaten, im inneren Teile von Pentosanen, Lipiden und Phosphatiden eingenommen wird. Wesentlich ist die stete Variabilität des Aufbaues während verschiedener Entwicklungsstadien. Die Vermutung liegt nahe, dass sich in dieser Erscheinung der Einfluss der $[H^+]$ bzw. $[OH^-]$ auf Hydratation und Permeabilität der Zellmembranen ausdrücken könnte. Auch Ülehlä sieht ja die Bedeutung der $[H^+]$ in der Veränderung der Membrankolloide (Dispersität, Hydratation). In jüngster Zeit stellt auch Mevius (p. 675) fest, wie bei niedriger $[H^+]$ die Permeabilität der Membranen für eindringende Salze stark erhöht wird, sodass eine

Überschwemmung der Protoplasten mit denselben erfolgen muss. Die Vermutung, die $[H^+]$ sei auch für die Anlage sklerotisierter Membranen bedeutungsvoll, kann durch diese und ähnliche Überlegungen schon *a priori* sehr an Wahrscheinlichkeit gewinnen.

Bei der *kausalen Analyse der beiden denkmöglichen Potenzen der sekundären Rinde*, die beide zu den eigentümlichen Sklerotisierungsphänomenen führen, dass die äusseren (älteren) Schichten reicher an sklerotisierten Elementen werden als die jüngeren, ist Pfeiffer (1925 a, p. 62) zu dem Resultat gelangt, dass sowohl die correlative Wirkung zwischen den Zellen der sekundären Rinde und die dadurch bewirkte "*sclerotio continuationis*," als auch die Determination von Sklerotisierungen bei diffus verteilten Gewebeelementen unabhängig von correlativen Einwirkungen, d.h. bei der "*sclerotio aetate ingravescente*" (scl. aet. progrediente)—ganz im Sinne von G. Klebs—auf *qualitative Zelldifferenzen* als die "determinierenden Komplementärbedingungen physiologischer Entwicklungsprozesse" (Pfeiffer, 1925 b) zurückgeführt werden müssen. Nachdem bereits lange vor Hansteen-Cranner vielleicht als erster de Vries einen Zusammenhang zwischen Zuckerbildung und Celluloseabscheidung angenommen hatte, ferner aus angestellten Untersuchungen eine innige Beziehung zwischen den oft als Membran der Sklerenchymzellen auftretenden Hemicellulosen (insbesondere Galactanen und Pentosanen; vgl. Grafe) und ihren hydrolytischen Produkten, den Pentosen, resultierte, wurde dargestellt, dass als zureichende Komplementärbedingung der Sklerotisierung von Rindenzellen die Gegenwart von Pentosen zu betrachten sei (Pfeiffer, 1925 a, p. 61). Die theoretisch zu fordernde Hydrolyse wurde dort der katalytischen Wirkung irgendwelcher Enzyme zugeschrieben, die der Hemicellulosen z. Bsp. der Cytase (H. T. Brown), wiewohl diese bislang nur aus Samenschalen und Pilzen dargestellt worden war; auch gelang es in der Folge nicht, sie im Gewebe der sekundären Rinde nachzuweisen. Es ist von Pfeiffer (*l.c.* p. 62) darauf hingewiesen worden, wie in der bestimmten Kohlenhydratkonzentration nicht das primär die Membranverdickung hervorrufofende Agens gesehen zu werden braucht. Unter Beachtung der Haberlandtschen Vorstellung von den Zellhormonen verschiedenster Art könnte die Pentosenstauung oder -Anhäufung event. nur die Summe (oder einen Teil dieser) der Realisationsfaktoren vorstellen, durch deren Verifizierung sodann die Möglichkeit eines Eingriffes der dann unbekannten determinierenden Komplementärbedingungen—vielleicht vom Charakter irgendwelcher Hormone—geschaffen würde.

Immerhin dürften die Tatsachen des *Zustromes von Kohlehydraten* aus der Umgebung und der *Notwendigkeit der Anreicherung mit diesen* im Cytoplasma der Gewebeelemente für viele Fälle als gesichert gelten, indem die Beobachtungen darüber ziemlich häufig zu sein scheinen und seit längerer Zeit bereits bekannt sind (Tischler, 1901). Wie in vielen Fällen bei beginnender Membranbildung und -Verdickung unverkennbar starker Verbrauch von Kohlehydraten zu konstatieren ist, wird auch von Czapek (p. 708) dargelegt. Von manchen Autoren wird nun angenommen, dass die Anreicherung mit Kohlehydraten nur die Versorgung mit Rohmaterialien für die seitens des Kernes produzierten hypothetischen "Gestaltungsstoffe" (Tischler, 1921-22, p. 352) bedeute. Die unbedingte Notwendigkeit der Existenz eines Zellkernes¹ zur Bildung der Zellmembran kann und soll hier allerdings in keiner Weise bestritten werden (vgl. Tischler, *l.c.* p. 237 sq.; Küster, 1924, p. 1021 sq.; Wisselingh, 1925, p. 230 sq.). Das Abhängigkeitsverhältnis von Caryo- und Cytoplasma, das in die Begriffe der "Kernplasmaspaltung" oder "Kernplasma-relation" gekleidet zu werden pflegt, mag man sich in der Weise verständlich machen können, dass Cytoplasma nebst Membran Reaktionsprodukte der chemischen Umsetzung von Kernproteiden sind. Da die Nucleine Kohlenhydratkomponenten enthalten [Pentosen], ist die Vermutung nicht unbegründet, dass jene als Baustoffe zur Membranbildung direkt beitragen können. Unter Beachtung der Wichtigkeit des Caryoplasmas für die Membranverdickung darf ferner das Faktum, dass die Mechanik jener wie die alles Zuwachses im allgemeinen zu einem erheblichen Teile von hydrolytisch aufspaltenden und kondensierenden Prozessen [Inversionen und Reversionen] beeinflusst wird, als unbestritten vorausgesetzt werden (Euler, p. 240 sq.). In der Natur von Inversionen und Reversionen liegt nun schon begründet, dass sie graduell weitgehend vom Wassergehalt und von der Lösungsfähigkeit der hydrolytisch zu spaltenden Salze bedingt sind. Zu der Verifizierung von Membranverdickungen gehört mithin ausser der Existenz des benötigten Baumaterials und sonstiger Komplementärfaktoren ein bestimmter Hydrolysegrad, den wir in stark übertragenem Sinne durch die [H⁺] ausdrücken können. Eine einfache Überlegung lehrt nun, dass in Rindenzenellen mit synthetisch stark aktivem Cytoplasma der Zuwachs an den Membranen

¹ Die Vorstellungen von der entwicklungsphysiologischen Bedeutung des Caryoplasmas bei den Phänomenen der Bildung und Verdickung der Membran (als Fermentproducent, Oxydationszentrum usw.) berühren die vorliegenden Untersuchungen indessen vorläufig in keiner Weise.

—ebenso wie der am Lumen—gewöhnlich recht bald sistiert wird, indem die Zellwand gegen Ausdehnung immer widerstandsfähiger wird und der ausdehnende Druck in demselben Grade abnehmen muss, in dem die kristalloiden Bausteine der Gewebeelemente zu Synthesen von Kolloiden und unlöslichen Zellbestandteilen verbraucht werden. Es liegt somit stets eine gewisse *graduelle Inaktivität der Rindenelemente* vor, wenn wir die Sklerotisierung ihrer Membranen beobachten.

Zwar möchte die weiter durchgeführte Analyse der von uns erörterten Gewebegeschehen in den pflanzlichen Sekundärrinden interessant genug erscheinen. Da es aber *verfrüht wäre, aller und damit auch der fast garnicht bekannten Faktoren zu gedenken*, so seien unsere Deduktionen an diesem Punkte abgebrochen. Es sei nur noch daran erinnert, dass nach Euler (p. 152) als Aktivator der von uns postulierten enzymatischen Pectatausfällungen in Zellwänden auch durch die Ca-Ionen ein Einfluss bisher nicht aufgeklärter Art ausgeübt wird. Es wird somit von uns nicht übersehen, dass die Sklerotisierung der Membran der Rindenzellen durchaus nicht von der $[H^+]$ allein abhängen wird, zumal sicher auch das Verhältnis der übrigen in der Lösung befindlichen Ionen an der Aktivierung beteiligt ist (vgl. Mevius, p. 676, und Abschnitt I. 1 dieser Abhandlung).

Die *Membranverdickung stellt also zum Teil das Resultat der chemisch synthetischen Inaktivität des Cytoplasmas dar* und ist als solches wiederum in hohem Grade einerseits von seiner eigenen Zusammensetzung abhängig, die wir bis soweit nur höchst unvollständig ahnen, andererseits ist sie von den äusseren Faktoren bedingt, welche die Reaktionsgeschwindigkeit nach einigermaßen bekannten Gesetzen beeinflussen: Licht (vgl. Wisselingh, p. 222) und Temperatur, $[O^+]$, Baumaterialien usw. Wenn auch unter den inneren Komplementärbedingungen, die zur Membransklerotisierung führen, nach den angestellten Untersuchungen im wesentlichen die $[H^+]$ bedeutsam wird, so muss doch die Überlegung, dass noch andere Komplementärbedingungen auf das Gewebegeschehen einwirken, zu dem Schlusse führen, dass das Cytoplasma je nach dem spezifisch zu definierenden Charakter und den aktivierten Morphosen äusserer Art in verschiedenen Fällen sehr ungleich potenziert werden muss, dass somit eine *unbedingte Proportionalität zwischen Membransklerotisierung und der $[H^+]$ nicht ohne weiteres zu erwarten sein wird*. So ist es auch zu erklären, wenn Starkly und Gordon zwar eine gewisse Proportionalität zwischen der Adsorption der Anionen und der Abnahme der $[H^+]$ konstatieren können, die Adsorption der Nitrate und Sulfate—im

Gegensatz zu denen der Phosphate—indessen nicht konstant von der Reaktion der Lösung beeinflusst finden.

Es bedeutet also ein immerhin etwas gewagtes Unterfangen, die Bedeutung der [H'] für die Verdickung der Zellmembranen in sekundären Rinden darzulegen. Einen gewissen Beleg für diese Einwirkung der [H'] mag man vielleicht indirekt darin sehen dürfen, dass sich die im ersten Hauptabschnitt dieser Abhandlung erwiesene Relation der [H'] zur Bildung von Calciumoxalatkristallen offenbar der Membranverdickung der Gewebeelemente proportional verhält. Doch darf nicht verschwiegen werden, dass bei solcher Eruiierung der wirksamen Komplementärfaktoren das Gewebegeschehen nicht analytisch bis in die letzten Einzelheiten überschen werden kann. Obzwar unsere angestellten Deduktionen also den Einfluss einer bestimmten [H'] wahrscheinlich erscheinen lassen, stehen wir doch noch vor der Aufgabe, diese Einwirkung genauer zu umschreiben und etwa mit dem isoelektrischen Punkte der Zellproteine in Beziehung zu setzen. Nachdem Pearsall und Priestley (1923, p. 189 sq.) die geringe Affinität der Proteine zum Wasser an ihrem isoelektrischen Punkte als eine der wirksamen Komplementärbedingungen für die Neubildung meristematischer Zellen innerhalb bereits differenzierter Gewebekomplexe erkannten und Herklots (1924, p. 250 sq.) zu ähnlichen Resultaten bei der Bildung von Wundmeristemen an Kartoffelknollen kam, musste die Vermutung auftauchen, dass für die Erscheinungen von Membransklerotisierungen eine andere [H'] massgebend sei.

Die Bedeutung des isoelektrischen Punktes der die Plasmamembran bildenden amphoteren Proteinsubstanzen für die Zellphysiologie, sowie die Tatsache, dass die physikalischen und chemischen Eigenschaften jener Proteine in Abhängigkeit geringster Differenzen in der Reaktion des Aussenmediums variieren, sind auch von Chodat erkannt, dessen gründliche Ausführungen (seine Einleitung) leider nicht mehr ausreichend verwendet werden konnten, ähnlich der kleinen Abhandlung von Gates. Gelegentlich seiner Diskussion der angeführten Theorie Priestleys hebt Weber (p. 292 sq.) als notwendige Stützen für sie hervor, dass bei der Zellteilung kolloidchemische Zustandsänderungen erfolgen,—er sieht sie vorläufig hauptsächlich in Viscositätsänderungen, die intime Zustandsabweichungen der lebenden Substanz verraten lassen—dass ferner Änderungen in der [H'] derartige Zustandsvariationen faktisch bewirken können. Einen grossen Teil der einschlägigen Literatur hat Spek in seinen Beiträgen zur Kolloidchemie der Zellteilung verarbeitet. Neuerdings sind Beziehungen des

isoelektrischen Punktes der Proteine zur Ionendiffusion in pflanzlichen Geweben durch Pearsall und Ewing aufgedeckt worden. Endlich sei auf die zahlreichen Literaturangaben Webers (p. 292) verwiesen. Die Tatsache einer Bedeutung des isoelektrischen Punktes der Membranproteine steht also weniger zur Diskussion als die spezielle Mechanik der ganzen Phänomene.

Selbstverständlich ist der *Sklerotisierungsprozess pflanzlicher Zellmembranen* in vielfacher Hinsicht abweichend von der Meristem-bildung; in mancher Beziehung mögen sich beide Vorgänge diametral entgegengesetzt verhalten. Zur Untersuchung des hypothetisch postulierten Zusammenhanges zwischen isoelektrischem Punkte der Proteine und Membransklerotisierung wurden Versuche mit den mehrfach verwendeten Indikatorenlösungen (Tropäolin oo, Dimethylgelb, Methylrot, Neutralrot, Phenolphthalein) angestellt, in deren Gebrauch durch die bereits dargelegten Untersuchungen immerhin eine gewisse Erfahrung gewonnen war. Schon die ersten, vorbereitend angestellten Anwendungsversuche ergaben für Rindenzellen mit wenig verdickten Membranen und kleinen, ausschliesslich monoclinen Kristallen eine relativ saure Reaktion, für Elemente mit sklerotisierten Wandungen und grossen Einzelkristallen des tetragonalen Modus oft einen dem isoelektrischen Punkte genäherten oder deutlich basischen pH -Wert. Unentschieden musste es gelassen werden, ob der Zusammenhang der $[H^+]$ mit der Kristallbildung oder mit der Membranverdickung hergestellt ist. Von den benachbarten Rindenzellen ist der Aciditätsgrad stets ein wenig verschieden, und es ist zu vermuten, dass gerade das Gefälle in der $[H^+]$ das wirksame Agens darstellt, aus dem die Phänomene der Membransklerotisierung resultieren. Überall dort, *wo sich die $[H^+]$ von dem isoelektrischen Punkte der Rindenproteine entfernt, dürften die Determinationsbedingungen zur Membranverdickung gegeben sein*, und je weiter der pH -Wert abweicht, desto energischer wird der Prozess einsetzen und ablaufen.

Am deutlichsten mussten diese Beziehungen in solchen Pflanzen zu erkennen sein, in deren sekundärer Rinde sich die mit sklerotisierten Membranen versehenen Elemente zu zusammenhängenden Komplexen sammeln und gegen die Gewebepartien mit unverdickten Zellwänden scharf getrennt bleiben. Ausser frischem Material von *Acer pseudoplatanus* L., *Betula urticifolia* Reg. und *Rhamnus alnifolia* L'Hér. kam eine von Luetzelburg (Nr. 15020!) in Brasilien gesammelte *Pithecolobium* spec. dieserhalb zur Untersuchung. Gegenüber ganz jungen Stadien von Zellen mit unverdickten Membranen ergab sich mit relativ deutlicher Genauigkeit ein Wechsel in der Acidität

derart, dass sich die Gewebeelemente vor der Anlage von Verdickungslamellen dem isoelektrischen Punkte der Rindenproteine nähern, während beim Einsetzen von Sklerotisierungen die [H'] einen mehr basischen pH-Wert aufweist. Ältere Stadien zeigen hinwiederum bei Anwendung der Indikatoren eine mehr oder minder saure Reaktion des Cytoplasmas. Gegenüber den benachbarten Gewebekomplexen mit unverdickt bleibenden Zellwänden bedeutet diese Variierung der [H'] die Entstehung eines Konzentrationsgefälles, zu dem die anders reagierenden Partien mit sklerotisierenden Membranen vertikal verlaufen. Die Untersuchung zahlreicher Querschnitte erweist mit wenigen Ausnahmen, die kein eindeutiges Resultat erkennen lassen, ein *Zuwachsoptimum für die Membranverdickung auf der alkalischen Seite des isoelektrischen Punktes der Rindenproteine*. Freilich kann das Optimum nach den angestellten Ermittlungen nicht konstant liegen. Nur wird durchgängig *gegen den Neutralitätspunkt hin ein starkes Fallen der Membranverdickungskurve* zu konstatieren sein [vgl. hiermit auch die Lage des Wachstumsoptimums nach Lindfors!].

Zur besseren Fundierung der vorgetragenen, vorläufig allerdings noch mehr oder minder hypothetischen Deutung müsste natürlich die Anstellung irgendwelcher Experimente erfolgen. Leider konnte bis soweit noch keine experimentelle Methode ersonnen werden, die Aussicht auf erfolgreiche Prüfung des Fragenkomplexes geboten hätte. Wenn die Untersuchung von Querschnitten nicht in *allen* mit völlig gleichdeutigem Resultat abgeschlossen werden konnte, so ist das zwar nicht verwunderlich¹, aber für die Darlegung der Hypothese doch sehr bedauerlich. Da mag denn wenigstens eine gewisse *logische Begründung* der erkannten Erscheinungen zur weiteren Stütze der Anschauung angeführt sein. Im isoelektrischen Punkte sind verschiedene physikalische Eigenschaften der Proteine im Minimum (z. Bsp. Quellbarkeit), andere im Maximum (Färbbarkeit usw.). Wo längs eines Gefälles in der [H'] der isoelektrische Punkt der haupt-

¹ Hierzu sei den weiter oben angegebenen Konsequenzen hinzugefügt, dass nach den bisherigen Erfahrungen die Acidität in gewissem Grade auch eine Funktion des Tageslichtes darstellt, indem sich jene proportional zum letzteren verringert. Die Arbeitsleistung der Pflanze bei der Adsorption aus einer Lösung geringerer Konzentration in eine solche von höherer kann, wie leicht ersichtlich, eben nicht ohne den benötigten Energieaufwand erfolgen. Die Energiequelle werden wir bei autotrophen Pflanzen natürlich direkt oder indirekt in optischen Kraftsystemen suchen (vgl. Hoagland und Davis, 1923). Auch mag die Temperatur für die Ionenaufnahme bedeutsam werden. Überhaupt dürften diese Phänomene *allgemein zu Prozessen des Wachstums und des Stoffwechsels, die Energietransformationen bedingen, in Beziehung stehen*.

sachlichsten Zellproteine liegt, wird das Cytoplasma in bestimmtem Grade die Tendenz aufweisen, Wasser an die Zonen anderer $[H^+]$ und grösserer Affinität dafür abzugeben. Aus der Wasscrabgabe wird in Gewebekomplexen anderer $[H^+]$ der Anstoss resultieren, die synthetischen Prozesse durch hydrolytische zu ersetzen (Weber, p. 292). Mit der *Abnahme der synthetischen Aktivität der Gewebelemente* sind also die bedingenden Faktoren für die Membransklerotisierung gegeben, sofern auch die andern Komplementärbedingungen einer solchen nicht widerstreiten. Es müsste daher etwa stattgefundenen Hydratation der cytoplasmatischen Kolloide (im Sinne von Borowikow oder Fischer und Hooker) rückgängig verlaufen. Die kausale Begründung dafür, weshalb die Zellkolloide in verschiedenen Entwicklungsphasen eine ungleich starke Hydratation und Solvation —denn wir haben auch mit einer chemischen Bindung der Eiweissmoleküle zu rechnen, nicht nur an einfache Quellungen zu denken— aufweisen, ist allerdings noch wenig durchsichtig¹. Es sind also *alle spezielleren Vorstellungen wohl noch zu früh*, um das ungleiche Verhalten der Membran- und Zellkolloide verstehen zu können.

Wenn wir zum Schluss das Resultat dahin formulieren, dass nicht nur für den meristematischen, teilungsfähigen Zustand von Gewebelementen eine bestimmte $[H^+]$ vorausgesetzt werden muss, sondern dass wiederum *durch eine ganz bestimmte $[H^+]$ auch das Einsetzen von Sklerotisierungsprozessen bedingt* wird, so soll in dieser allgemeinen Fassung eben unentschieden bleiben, ob für die beiden Phänomene der Gewebephysiologie faktisch nur der isoelektrische Punkt von Proteinen als dem Maximum der Entquellung der cytoplasmatischen Kolloide charakteristisch ist, oder ob nicht vielmehr gerade das *komplizierte Zusammenwirken der verschiedenen Proteine*, von denen jedem ein besonderer isoelektrischer Punkt zugeschrieben werden muss, von ausschlaggebender Bedeutung ist. Sollte sich die letztgenannte Möglichkeit verifiziert finden, so würde die gesamte Zellphysiologie—wie mehrfach erkannt worden ist—dadurch ganz ungeheuer vereinfacht sein, indem bei einer einzigen $[H^+]$ alle Abstufungen des Quellungsgrades und aller andern physikalischen und kolloidchemischen Eigenschaften an den einzelnen Molekülen des Cytoplasmas gleichzeitig zur Auswirkung gelangen könnten. Eine Fülle weiterer Fragen, deren Lösung späteren Untersuchungen vorbehalten bleiben muss, tritt uns in jedem Falle entgegen. Wenn

¹ Am sichersten begründet scheint mir im Augenblick die Ansicht, dass die Hydratationen durch Oxydationsprozesse mächtig gesteigert werden können; vgl. Bechhold, p. 287 sq !

manches wichtige Problem hier noch nicht berührt worden ist, so möge man diesen Nachteil mit dem *Missverhältnis zwischen der Weite des ganzen Problemkomplexes und der Beschränkung der Zeit und Arbeit*, die ich ihm zu widmen vermochte, entschuldigen.

SUMMARY.

I.

As the result of the study of crystallization of calcium oxalate in the presence of media of different hydrogen-ion concentration, the following conclusions are drawn:

1. The process of crystallization is determined at most partly by the hydron concentration of the solution.

2. Experimental grounds are given for the presumption that the monocline crystalline form is assumed at low pH values while the tetragonal and the "gland" forms are found at neutral or basic pH values.

3. The influence of hydrogen-ion concentration is especially conditioned by the combination of the dissolved calcium salts.

4. The use of certain indicators in the anatomical observations on the secondary cortex of plants has shown that "Neutralrot" fixes the degree of acidity which is decisive for the origin of tetragonal crystals. Butter-yellow ("Dimethylgelb") and "Methylrot" can be used as a criterion for pH values from which monocline crystals result.

5. In the secondary cortex of plants the actual acidity shows a maximum on the acid ($pH = 2,4-3,8$) and another on the basic ($pH = 7,2-8,6?$) flank of the protein iso-electric point.

6. It is suggested that the methods which can be used to determine the different hydrogen-ion concentrations of the cytoplasm in living plant tissues have to surmount many difficulties.

7. The actual acidity of the cells of the secondary cortex, especially during its development, expressible as hydron concentration, is continually changing.

8. It is an open question whether the optimum of crystallization is displaced by $CaCl_2$ to the acid flank of the iso-electric point of the tissues.

9. The zonal distribution of the crystals of calcium oxalate is probably not reducible to hydron concentration but to the phenomenon of Liesegang's rings.

II.

In the second part of this communication the plant cortex is regarded as in dynamic equilibrium between internal and external factors among which hydron concentration is considered. In completing Priestley's (1923) theory the following conclusions result:

10. Before sclerization of membranes the streaming of carbohydrates from the surrounding tissue and the enrichment of cytoplasm with nutritive substances—corresponding to H. Pfeiffer's (1924, 1925 *a*) working hypothesis—take place.

11. The sclerizing process is partly the result of the synthetic inactivity of the cytoplasm, and is therefore also determined by the hydrogen-ion concentration.

12. An absolute proportionality between thickening of cortical membranes and the actual acidity is not always to be expected.

13. But it is beyond all doubt that the cells of a thin-walled plant cortex show a somewhat acid reaction, while the sclerized elements of cortical tissue possess a more or less alkaline *pH*-value.

14. In adjoining cells the degree of acidity may vary. Hence the fact that the sclerized cells lie across gradients of hydron concentration suggests that they occur at the point where the actual acidity deviates from the iso-electric point of important constituent cortex proteins.

15. The degree of this deviation indicates the vigour with which the process is carried out.

16. Probably we have to assume an optimum region for wall thickening on the alkaline flank of the iso-electric point. Of course this optimum is not constant, but towards the point of neutrality a rapid fall of the curve of wall-thickening is to be noticed.

17. The phenomena of sclerization are certainly called into existence at a well-defined hydrogen-ion concentration of the cell wall proteins.

18. In this physiological process the iso-electric point may determine the maximum precipitation of cytoplasmic colloids. But probably the complicated co-operation of the different proteins with their varied iso-electric points is more significant in the physiology of plant tissues.

BREMEN (GERMANY),

12 March, 1925

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SUGGESTIONS CONCERNING THE ABSORPTION OF IONS BY PLANTS¹

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STILES⁽¹⁾ has recently published a comprehensive review dealing with the subject of permeability. A perusal of this work, while indicating the very extensive consideration which has been given to this field of research by numerous investigators, also emphasises the great diversity of opinion held concerning the nature of the phenomena involved in the penetration of substances into living plant cells. The term "permeability" has been used in many ways, but the majority of investigations cited by Stiles have been conducted with solutions of relatively high concentrations, or with solutions of organic compounds not occurring in the normal nutrient media of the higher plants. During recent years, a considerable interest has developed in what may be called the nutritional aspects of the subject which have to do with the absorption of mineral elements from solutions of relatively low concentration, as related to plant growth. The great majority of investigations on permeability have thrown very little light on these relations which must, nevertheless, be regarded as of greatest importance, since their elucidation is indispensable to the development of researches on the growth of plants in soils. During recent years, much has been learned about the soil solution with regard to its concentration and composition, and its dynamic nature. The absorption of ions from solutions resembling soil solutions is, therefore, a field of inquiry which possesses both physiological and agricultural interest. It is possible that the readers of the review by Stiles may be interested in a brief statement concerning some of these problems, based on the results of numerous researches carried on in the laboratory with which the writers are associated. Many of the results of the researches are not susceptible of interpretation in terms of cell permeability, if this word be defined in its strictest sense, and, for this reason, we prefer to discuss our views under the title of the absorption of ions by plants. It is, of course, obvious that such absorption is, in part, dependent on cell permeability, but the determination of the extent of absorption

¹ Revision of a paper read at the meetings of the British Association for the Advancement of Science, Section M, Liverpool, 1923.

of a substance is not necessarily equivalent to a determination of the permeability of the cell for that substance. This point of view has been made clear by Osterhout(2) and others. On the other hand, many problems of plant nutrition require a careful consideration of the actual intake of ions as affected by the concentration, composition and reaction of the culture solution and by the aerial environment of the plant.

Not infrequently, the impression is given that the absorption of ions by plants is in some way related to the process of osmosis. According to the views of the modern chemist, as expressed, for example, by Washburn(3), osmosis refers to the passage of a solvent from one side of a semi-permeable membrane to the other, when the escaping tendency (or free energy) of the solvent on the two sides is unequal. The osmotic pressure is defined by him as "the pressure difference which must be established upon the solution and pure solvent, respectively, in order to make the escaping tendency of the solvent the same from both of them." Osmotic pressure then, is not to be confused with diffusion pressure which the same author defines as the "partial pressure which the solute molecules in any solution exert against a membrane permeable only to the solvent." This latter is a real pressure existing within the solution, the limiting value of which depends upon the thermodynamic environment, the former, on the other hand, is an abstraction in the sense that it is independent of osmosis, semipermeable membranes, or molecular theory. Diffusion pressure entered into the older theories of the plant physiologists in which they invoked the bombardment of solute molecules as an explanation of osmotic pressure. Obviously, neither of these two pressures can account for the passage of solutes through a cell membrane, and, with the definition of osmosis given above in mind, it would not be desirable to employ this term in explaining the passage of ions into a plant cell.

Certain of the essential conditions governing the absorption of ions by a plant cell can be presented most clearly by reference to some very recent experiments on the fresh water alga *Nitella* (Osterhout(4), Irwin(5), Brooks(6), and Hoagland and Davis(7,8)). This alga produces cells so large that the vacuolar sap may be obtained from individual cells in an almost uncontaminated condition. When this sap is analysed, it is found that the important inorganic elements are present in concentrations many times greater than those occurring in the pond water from which these elements are taken. For example, in our experiments, we found only a trace

of potassium present in the outside medium, at the same time that the cell sap contained this element in a concentration of approximately two thousand parts per million. The concentration of chlorine in the sap was a hundredfold greater than in the surrounding pond water. A further examination of the sap showed that only very small amounts of organic matter were present and that nearly all of the inorganic elements or radicals must have existed in dissociated form in order to account for the electrical conductivity as determined. These findings are quite in accord with the older work on animal cells by Höber(9) who determined the conductivity of solutions within living cells by placing the tissues in the axis of a coil through which a rapidly alternating current was passed. The diminished strength of the current due to the damping of vibrations was stated to be an index of the internal conductivity of the cell, which was computed to be that of a tenth normal salt solution.

We have, in the case of the *Nitella* cells, a striking illustration of the diffusion of ions from a solution of low concentration into a solution of higher concentration. The relative distribution of ions is also very different in the two solutions. Now it is essential to assume that such diffusion and distribution of ions can come about only by virtue of work done within the plant cell, if the second law of thermodynamics is to apply in such cases. We have carried out some very simple experiments with *Nitella* which may, perhaps, have a bearing on the energy relations of absorption. Equal masses of healthy cells were placed in slightly acid solutions of $\cdot 001$ m. KCl. Some cells were kept in the dark, some in a dim light, and others exposed to varying periods of illumination, *i.e.* one hour, three hours, five hours, eight hours, and the entire period of daylight. After a few days, the amounts of chlorine removed from solution were determined, and there was found to be a very definite general correlation between absorption and illumination. Only very slight absorption of chlorine occurred in the complete absence of light, or when the periods of illumination were very short. It might be true, of course, that permeability is directly affected by illumination, but it seems more probable that the energy derived from the sunlight is indirectly involved in the absorption processes. Energy required by root cells for those purposes would, of course, be derived from carbohydrates synthesised in the green parts of the plant. As might be expected, the existence of the condition which has just been described, is dependent upon the normal functioning of the cell. For example, in *Nitella*, even a slight disturbance of the normal life processes may

cause chlorine ions to diffuse out of the cell into the surrounding solution. This does not appear to take place so long as the plant is uninjured, or its normal metabolism remains undisturbed. In a word, these living plant cells seem to possess the property of one-way permeability. From these considerations, we must assume that the solutions inside and outside the cell are not in any true chemical or diffusion equilibrium, and that the conditions cannot be explained by a simple application of the law of mass action although the latter may be involved in certain stages of the processes of absorption. Furthermore, a cell, such as has just been described, obviously cannot present a simple case of a Donnan equilibrium. Mass action relations, Donnan effects, protein isoelectric points, may all be, and probably are, of utmost importance to the functioning of the cell, and yet fall far short of explaining the final distribution of ions.

It seems essential to recognise that plants, and no doubt, living cells in general, have the power to bring about the movement of solutes against a concentration gradient, and that this phenomenon is not limited to certain definite organs, such as the kidney in the animal body. While energy expenditure must be postulated as being involved in these processes, there is no reason for assuming that some special or "vital" form of energy would be required. The mechanism by which absorption from lower to higher levels takes place, or by which higher concentrations are maintained within the cell, is still an unsolved problem. One of the most obvious suggestions is that differences of electrical potential must be taken into account, but in the absence of further definite experimental evidence, such speculations lead only to vague ideas¹.

While the illustration of the *Nitella* cell relates to a plant of relatively simple type, it is entirely logical to assume that the general principles governing absorption of ions by plant cells are not dissimilar in the higher plants. It is unfortunately impossible to obtain uncontaminated cell sap from such complex organisms, but the observations which have been made on the expressed juices are indicative of a condition which is quite analogous in several respects to that which exists in *Nitella*. (In a recent article Pearsall and Ewing⁽¹⁰⁾ also suggested a similar view.) The tissue fluids expressed from the leaves of barley plants grown in an ordinary culture solution

¹ With regard to the general question of absorption or secretion against a concentration gradient, see also discussions by Bayliss in *Principles of General Physiology* and by Benjamin Moore in *Biochemistry*. In connection with plant cells, a similar point of view has been incidentally suggested by Pantanelli (*Bull. Orto Bot. Napoli*, 6, pp. 1-37, 1918).

have conductivities very much greater than those of the solutions from which the plants obtain their inorganic elements. The distribution of ions in the tissue fluids when considered in connection with the conductivity measurements, leads to the conclusion indicated in the first part of this paper, that a large proportion, at least of several of the important inorganic elements, is usually present in dissociated form. Potassium ions in equilibrium with organic acid anions (and under certain conditions with nitrate ions) are especially important in this system. Various experiments with very different types of plants have proved that practically all the potassium present in a plant is easily soluble in water, and no evidence of the existence of any organic combination, except in the case of a very small proportion of the potassium, has been adduced.

With regard to the roots, the total ion concentration of the tissue fluids, while smaller than that found in the leaves and stems, may still be much greater than the ion concentration in the surrounding solution.

The selective action of plants is a characteristic which is universally recognised and yet it would appear that many erroneous ideas have been held concerning this question. While it is undeniably true that the plant may absorb certain ions much more readily than others, it is not true that a plant necessarily selects from the solution only, or even chiefly, those ions which are indispensable to its growth. This older conception of selective action has been expressed in such statements as the following: "Plants absorb NO_3 readily from sodium nitrate, but only a trace of sodium," or, "Potassium is readily absorbed from potassium chloride, while the chlorine is absorbed only to a slight extent." As a matter of fact, experiments made with barley and with other common agricultural plants, show that many unessential ions (unessential at least in more than minute quantities) may be absorbed very readily. Chlorine and sodium may be cited as examples. It was found that chlorine was absorbed much more readily than sulphate, although the latter is an essential ion, the behaviour of bromine being very similar to that of chlorine. Sodium may be absorbed by barley much more rapidly than calcium from some solutions. These results are consistent with the observations of Stiles and Kidd(11), and of various other investigators.

It has been shown by Pantanelli(12) and by others that the two ions of a salt are seldom absorbed in equal proportion, but any large absorption of one ion in excess of that of the oppositely charged ion in equilibrium with it necessarily depends upon what, in effect,

amounts to an exchange of ions. It is true that when plants are transferred immediately after germination, or after only brief contact with complete culture solutions, to dilute solutions of K_2SO_4 , as well as to solutions of a number of other salts, a distinct increase of hydrogen ion concentration may occur (for example, from pH 5.0 to pH 3.2), but this process is self-limited, so that the total quantity of potassium ions in excess of sulphate ions which can be absorbed in this manner is very small. When any considerable excess of potassium ion is removed from solutions of single salts, other cations, especially calcium and magnesium, will be found to have been displaced from the root tissues, possibly from some constituents of the cell wall. The increased acidity produced in potassium sulphate solutions cannot be attributed to the specific need of the plant for potassium, since a similar increase of acidity frequently occurs in sodium sulphate solutions. In the case of the nitrate ion, the anion may be removed from solution far in excess of the cation, such excess removal, as shown by barley plants, being especially pronounced when calcium is the cation (13). This process is made possible by the ease with which NO_3 ions are replaced in the solution by HCO_3 ions, either directly or indirectly. In this way, carbon dioxide production by the roots may play an important rôle in the absorption of ions by plants. It is of interest to note that not only NO_3 ions, but also chlorine ions may be replaced by HCO_3 ions, although to a much smaller extent. The latter type of replacement was observed when vigorously growing barley plants were transferred to dilute solutions of $CaCl_2$. In our experiments with alkaline culture solutions, cations were absorbed to a greater extent than the anions present (other than OH , HCO_3 , and CO_3), and the OH ion concentration of the solutions was decreased. In complete culture solutions containing nitrate, both alkaline and acid solutions in most cases tended to have their reaction changed to a point very close to that of neutrality. The reaction which is attained under these circumstances, appears to depend primarily on the equilibrium between CO_3^{--} , HCO_3^- and CO_2 . These observations do not preclude the absorption of HCO_3 ions, which may readily occur, for example, from a solution of $KHCO_3$.

From what has been said in the foregoing paragraph, it is evident that the ions of a solution may be exchanged in unequal degree for other ions of the same charge, derived from the plant, but likewise important is the fact that one ion may either retard or accelerate the absorption of another ion. For example, the slow rate of pene-

tration of sulphate ions decreases the rate of intake of potassium ions, so that with solutions of equal concentration, potassium is absorbed by barley plants much more rapidly from the chloride than from the sulphate(13). This is an example of the influence on each other of ions of opposite charge and may, perhaps, be thought of as an electrostatic effect. Cations may also be interrelated in the processes of absorption; thus a relatively high concentration of sodium may depress the absorption of potassium or calcium. Anions likewise may show similar interrelations. When complex culture solutions are used, the intricacy of the problem will at once be apparent, but a satisfactory understanding of the absorption of ions from such solutions must take into consideration the relations of anion to cation, of cation to cation, and of anion to anion. In connection with the first relation, it is possibly justifiable to suggest that the rapid intake of nitrate and potassium ions may increase the rate of intake of other ions. The earlier work of Waynick(14), the recent work of Reed and Haas(15), and that of one of the writers(16), all give direct evidence of the importance of considering the effect of one ion on another in any attempts to formulate hypotheses or theories regarding the removal of inorganic elements from soil solutions or artificial culture solutions.

The interrelations of ions now under discussion pertain in general to solutions of concentration which are met with very generally in culture solutions and in soil solutions. Since in the majority of experiments on antagonism, solutions of higher concentrations have been employed, the question arises whether similar effects can be observed at these low concentrations. Osterhout(3) states that in such dilute solutions, phenomena of antagonism may become relatively unimportant. A solution of a single salt, such as KCl, may not possess toxicity to a plant at a low ($\cdot001$ to $\cdot01$ or $\cdot02$ molar) concentration, but may produce marked injury at a higher concentration, which injury can be prevented by the addition of another salt of suitable character. In the latter case, the mixture of salts preserves normal permeability; on the other hand in dilute culture solutions, or in ordinary soil solution, antagonism, as usually understood, may not be of any great importance. It is possible, however, even in very dilute solutions, for one ion to affect the rate of penetration of another ion. Referring again to the experiments made on *Nitella*, it was found that the rate of penetration of NO_3 ions into the cell sap was significantly depressed by the presence of chlorine ions in the solution. In connection with plant nutrition investiga-

tions, special interest attaches to the determination of the absorption of individual ions, rather than to net changes in electrical conductivity. For example, the latter measurement might indicate increased permeability in the presence of a considerable concentration of sodium salts, while chemical analysis might point to an increased absorption of sodium ions, but a greatly diminished absorption of calcium ions, with significant consequences to plant growth. It is clear that in such cases, it is not sufficient to determine that general alterations of permeability have occurred. In studying the growth of the plant as a whole, we must ascertain for each individual ion whether its rate of absorption is being increased or decreased.

The relation of the absorption and transpiration of water to the absorption of ions has interested numerous plant physiologists, and many contradictory ideas are held, as has been well pointed out by Muenscher(17) in a recent article. It can be safely asserted, however, that inorganic elements may be absorbed by a plant more or less independently of the absorption of water. Our own results indicate that ions do not all show the same behaviour in regard to the differential absorption of ions and water. During a given period of plant growth, the same solution may become much more concentrated with respect to some ions and much more dilute with respect to other ions. In brief, water may be absorbed either more or less rapidly than the ions present in the solution, depending on the conditions of growth and transpiration, the concentration of the solution, and the nature of the ion. It is equally incorrect to consider the plant either as an organism carefully selecting only the essential ions from the culture medium, or as a sort of wick, taking up the solution, evaporating the water, and leaving the solutes behind. A plant may obtain an adequate supply of a solute found in the culture solution in low concentration (such as PO_4) without a proportionate absorption of water. On the other hand, it is not safe to state that a plant cannot, under some circumstances, benefit by an increased supply of certain ions, which may be connected with increase of transpiration and of water intake, leading to an increased rate of movement of water from roots to tops. Unfortunately, it is difficult to distinguish between the direct effects of increased water intake and of increased growth and accelerated metabolism, which may often accompany increased transpiration.

The general effects of the hydrogen ion concentration of the medium on the growth of plants has been investigated frequently during the past few years, but very little work has been carried out

on the relation of the reaction to the actual intake of different ions. Some years ago, one of the writers⁽¹⁸⁾ obtained some preliminary results bearing on this point and more recently Theron⁽¹⁹⁾ and also the writers have continued to give attention to the question. (Compare also recent researches by Pearsall and Ewing⁽¹⁰⁾.) The evidence now at hand gives considerable assurance that the reaction of the solution may become a very important factor in ion absorption. This may be illustrated by certain experiments performed on *Nitella*. It was found, for example, that nitrate ions penetrated into the cell sap much more readily from an acid solution than from an alkaline one. Chlorine ions, also, were removed more readily from an acid solution than from an alkaline one. Similarly, barley and cucumber plants removed a greater proportion of nitrate from a solution with an acid reaction (pH 5.0), than from one in which the reaction was slightly alkaline, other conditions being essentially the same. From an alkaline culture solution, a relatively greater proportion of cations was absorbed by the plants just mentioned. Our earlier impression was that a plant tended to change the reaction of the culture medium to a point optimum for growth. Such is not the case with a number of single salt solutions (K_2SO_4 , $(NH_4)_2SO_4$, etc.), nor with complete culture solutions containing nitrogen in the form of ammonium ion, where the reaction may be changed by the growth of some plants to pH 3.2. Even in the complete culture solutions containing nitrate, the reaction of pH 6.6–7.0, so often obtained as a sort of equilibrium point (when the phosphate buffer is not too great), is not of necessity the optimum, since, in some experiments, it has been found that an appreciably more acid reaction, if maintained, produced superior growth of a number of species of plants during the earlier stages of growth at least.

While the reaction of the medium appears to influence the rate of absorption of certain ions, it does not follow that there exists any simple relationship between the reaction of the outside medium, the reaction of the cell sap, and the removal of ions from solution, especially on the supposition that elements like potassium undergo a precipitation or combination within the cell sap. Furthermore, it has not been possible to show that any simple relation exists between the reaction induced in a culture solution by plant growth and the isoelectric points of the proteins present in the plant. This, of course, does not mean that the isoelectric points of plant proteins may not play an important rôle in plant metabolism, as suggested in various recent articles.

It is of interest to note in this general connection that our experiments on *Nitella* indicated that the hydrogen ion concentration of the sap of normal cells remained at a practically constant value ($pH\ 5.2$), even when the cells were immersed in solutions of widely different reactions. The preservation of a definite reaction in the vacuolar sap may be essential to the normal functioning of the cell. Tissue fluids expressed from higher plants may not always show this condition so clearly, but such tissue fluids represent a very complex mixture obtained by crushing both living and dead tissues, and cells of many different types are involved. There is considerable evidence that different types of cells may have different reactions. The reactions of the sap expressed from a mass of tissue may not, therefore, represent the reaction of any particular cell. Incidentally, it may be remarked that the reaction of the cell sap and of the protoplasm are not necessarily identical.

Intimately related to the absorption of ions is the question of ion or salt proportions in culture media. The writers, as well as others, have previously expressed the opinion that equally good growth of plants may be obtained from many culture solutions of widely differing composition. Recently, definite proof in favour of this position has been brought forward by one of us (20), who has shown that, taking into account variability, very different solutions may be equally effective, as measured by plant production. It is true that certain extreme types of solutions may cause a decrease of yield, but in this connection, more recent experiments in extension of the above mentioned investigation are of significance. It was shown in these that the relative yields from so-called "good" and "poor" solutions varied from month to month. At certain seasons, the poor solution produced plants equal to those produced by the good one, while at other seasons, a large difference between the two solutions was found. We cannot conclude, however, that the same absorption of ions will take place from different solutions; on the contrary the absorption will vary (but in no simple or direct way) with the composition and concentration of the solution, bringing about marked alterations in the composition of the plant with respect to inorganic elements. That is to say, two solutions may produce plants of equal dry weight, but of very different composition. Alterations may be made even in the composition of the seed, although ordinarily they are, of course, less marked than in the case of the stems, leaves, or roots.

It is evident, therefore, that many solutions of very different

composition can produce equally favourable plant growth, and, on the other hand, the same solution may be an excellent medium for the growth of many diverse types of plants. A number of years ago, we described a culture solution made up to imitate, in its ion proportions (except for phosphate), the extract of a certain fertile soil then under investigation. The total concentration was about 1500 p.p.m. This solution has since been employed in many experiments and it has been possible to obtain either in solution or sand cultures, very satisfactory growth of barley, wheat, alfalfa, peas, corn and Bermuda grass (*Cynodon dactylon*), as well as of numerous other diverse species of plants. Reed and Haas⁽¹⁵⁾ have been successful in growing citrus trees in a similar solution, in sand culture. It is, perhaps, unnecessary to state that in very deficient or toxic solutions, different types of plants will react in characteristic manner. Thus Bermuda grass will grow well in a solution with an extremely low concentration of calcium, which would be entirely unsuitable for common agricultural plants.

If it be acknowledged that the plant is not dependent upon a culture solution of narrowly restricted ionic proportions, it may then be asked whether certain definite concentrations of ions are required. Here, also, it is found that wide variations are permissible. The plant can adjust itself to solutions of relatively low concentrations by removing from them a greater proportion of the ions present than would be the case were a more concentrated solution involved. Obviously, the concentration might become so low that the plant would be unable to absorb in a unit of time the required quantity of the ion in question. Consequently the rate of absorption, as influenced by the reserve or renewing power of the culture solution or soil solution, becomes an important consideration. It is essential that withdrawals by the plant shall not lower the concentration below the critical point too early in the growth cycle. These critical concentrations may be expected to vary, not only with the ion involved, but with varying conditions of light, temperature, and humidity, and with the stage of growth of the plant. In the soil, additional factors will affect the rate of absorption, such as the extent of the root system and the intensity of carbon dioxide excretion by the roots. The latter factor will tend to modify the medium by increasing the rate at which unavailable compounds are rendered available. The relative importance of these factors remains to be determined.

At this point, reference should be made to the question of specific

absorbing powers of different plants. To what extent does a given plant species possess a characteristic composition? It is evident that this question is not capable of solution on the basis of data obtained from ordinary soil experiments, since the effective culture solution is unknown, and, as has been stated earlier in this paper, the composition of the medium is one of the primary considerations involved in determining the composition of the plant. Very few experiments are on record in which different types of plants have been grown in identical culture solutions and the absorption of ions compared. It can scarcely be doubted, however, that different species of plants may be found which will absorb different proportions of ions from the same culture solution, but no adequate idea of such differences can be formed from data now available. It seems safe to assert that in many cases, the composition of the artificial culture solution, or of the soil solution, as well as climatic conditions, will have a more profound effect on the composition of crops than differences in specific absorbing powers of the plants themselves.

On the basis of the comments made in this article, a number of general suggestions may be advanced pertaining to future investigational work on this phase of plant nutrition. In the first place, it may be questioned whether the greatest progress will be made by observations of the effects of many solutions of slightly varying composition on the yield of different plants, for the reasons which have already been stated. The effects of hydrogen ion concentration on the growth of plants is a matter of great interest, but to determine merely the pH values at which good growth can be obtained is, after all, only an initial step. In the field of plant nutrition, many of the most important problems have to do with the chemical processes concerned in the absorption and utilisation of ions. What purposes do these ions serve in the plant? Just how, for example, does potassium influence the transformations of organic constituents? What are the relative parts played by the different cations in the buffer system of the plant? What is the importance of ion-protein relations in the plant cell? These and many other similar questions come readily to mind when any review is made of our present state of knowledge of the functions of the essential elements. It is clear that an adequate understanding of the absorption of ions by plants awaits the greater development of the field of plant biochemistry, which must always keep in view the possible application of the recent discoveries of the physicist and chemist with regard to structure and properties of the chemical elements essential to plant growth.

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THE VARIATION OF LEAF FORM IN *POTAMOGETON PERFOLIATUS*

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(With 1 figure in the text)

AQUATIC plants possess several features which make them of great value to the student of form variation. Not only are they in many cases characterised by great variability in the form of their organs, but the habitat conditions under which they grow are much more uniform than is usually the case for terrestrial plants. In addition, submerged aquatics are particularly attractive to the experimental morphologist because, in the absence of transpiration and its attendant complications, the environment is simpler and comparatively easy to control. Few submerged species show greater variability of leaf form in nature, than *Potamogeton perfoliatus*. So much is this the case that taxonomists almost invariably split the natural forms into a number of sub-species or varieties (cf. Hagstrom(2)). The leaf variation is chiefly in the relative breadth of the leaves and it is most easily expressed as the $\frac{\text{length}}{\text{breadth}}$ ratio. This ratio varies, at least, between 1.5 and 6.0 in specimens from natural habitats, and the different types of leaf are usually of rather different texture and colour, the narrower ones being greener and thinner. The narrow leaves are also normally developed on stems which are thin and which possess much longer internodes. There is, however, no constant relation in natural specimens between the relative leaf breadth and the length of the internodes. In the variations indicated above, *P. perfoliatus* is representative of most submerged species of *Potamogeton* (and indeed, of many other genera of water plants) and the experimental analysis attempted below probably applies equally well to other species. Details of some of the natural forms are given in a previous paper (Pearsall(5)) and also by Glück(1) and Hagstrom(2).

Some authors associate the narrower leaved forms with their occurrence in running water. That the movement of the water cannot be the real causal factor is indicated by the fact that almost the whole range of forms in this species can be found in standing water, as for example in the English Lakes. In these lakes, the narrower leaved

forms usually occur in deeper water and hence in low light intensities. Thus specimens from 6 m. of water in Windermere, have a length/breadth ratio of 4.1, while this ratio is only 2.4 for specimens from water of 3 m. depth (Pearsall, *loc. cit.*). On the other hand, in other lakes, specimens with a high length/breadth ratio may be found in higher light intensities than in the case cited, and in some lakes, even in shallow water, the more roundly ovate leaves are never produced. These facts clearly indicate that some other factor besides light intensity is operating to produce the observed leaf variation in this species. Experiment confirms this view. Three sets of plants bearing the extreme types of leaves (average L/B : 1.8, 2.0, 5.5) were grown in tanks on the same garden soil and under uniform light conditions, the depth of the water being 1.5 m. The first formed leaves tended to resemble the original forms—but by the end of the summer (1920) all the plants were producing similar leaves, averaging 3.0 cm. in length and 1.55 cm. in breadth, *i.e.* with a L/B ratio of about 2.0. The following summer, these plants were grown in different light intensities, the same, half and one-fifth of those used the previous year. These were approximately 20, 10 and 2 per cent. of full sunlight. The same type of leaf was produced in the original light intensity, but the plants in half this light intensity had leaves 3.3 cm. in length and 0.9 cm. in breadth. The plants in the weakest light grew little and had much longer internodes, the leaves averaging 1.5 cm. in length and 0.4 cm. in breadth. In the last example, therefore, the length/breadth ratio was similar to that from half light intensity. It was further apparent that any further reduction of light intensity would lead either to no growth or else to very small completely etiolated shoots. This experiment left no doubt that some other factor besides light was involved in the leaf variation of *P. perfoliatus*. Although part of the material we started with had leaves between 5.5 and 6.0 times as long as broad, we had only been able to produce leaves averaging 3.66 (and at most 4) times longer than their breadth.

In the above experiment the original environments had been altered in at least one obvious respect. A fertile garden soil had been substituted for the different soils on which the different forms originally grew, and this might have affected the leaf variability to some extent. An examination of the limited number of soil analyses available showed that the leaf form in nature appeared to vary with the lime content of the soil (as indicated below), the broader leaved forms occurring on the more calcareous soils (Pearsall, *loc. cit.*).

TABLE I

Lake	Average leaf L/B	Available in soil*		Ratio K ₂ O/CaO
		K ₂ O	CaO	
Coniston	4.5-5.5	338	90	3.7
Ullswater	2.6	336	280	1.2
Esthwaite	1.8	342	1600	0.2

* Parts per million of dry soil.

No significant connection between the nitrate or phosphate content of the soils and the leaf form could be traced. The relation indicated in the table between the calcium content of the soil and leaf form was partly confirmed by the fact that all natural specimens available from calcareous districts had the broader type of leaf. The question was therefore examined experimentally.

The experiments were carried out by the method devised by Pond (6). The rhizome was enclosed in a stoppered water-tight bottle from which a tube led to the surface of the external solution and thus allowed the liquid inside the bottle to remain at uniform pressure and volume. The plants and bottles were submerged in cubical glass tanks about 2 feet in diameter placed in front of a north window. The material used was taken from the river Wharfe and the leaves had an average length/breadth ratio of 1.94 ± 0.20 . Equal lengths of rhizome each containing one young growing shoot were put into each of the experimental bottles. Very satisfactory growth was made in Shive's optimum three salt solution (7) diluted to one-tenth with Leeds tap water (which contains little calcium), and this solution was therefore used as the culture medium throughout. The experimental condition described below as "excess calcium" was obtained by adding about 5 gm. per litre of calcium nitrate to the culture medium. "Excess potassium" was obtained by adding a similar quantity of potassium di-hydrogen phosphate or potassium nitrate. The latter produced less shoot growth than the former, but the leaf form was similar in each case. The average lengths and length/breadth ratios are given below for the 1923 experiments:

TABLE II. Effect on leaf form of different culture treatments

Predominant basic ion in solution surrounding		Average leaf lengths (mm.)	L/B	No. of leaves measured
(i) Roots	(ii) Shoots			
1. Calcium	Calcium	22.0	1.72 ± 0.18	24
2. "	Potassium	24.1	1.79 ± 0.10	14
3. Potassium	"	22.9	3.38 ± 0.62	25
4. "	Calcium	25.2	2.26 ± 0.13	19



Fig. 1 *Potamogeton perfoliatus* from the same parent plant after excess K

Similar results were obtained in 1922. It is to be noticed that the experimental treatment produces little or no alteration in leaf length—only the relative breadth is altered. The extreme differences shown by Nos. 1 and 3 of the above table are illustrated in Fig. 1, which shows also the longer internodes possessed by the narrow-leaved plants. In the illustrations, the largest shoots are those last produced, and those showing the most striking difference. The effects are clearly produced chiefly by materials absorbed by the roots. If the latter are in "excess potassium" then a pronounced effect is still obtained when excess calcium is added to the solution surrounding the stems. An interesting additional case is that of control plants grown in the Shive's solution diluted to one-tenth, which was not renewed during the experiment. The first leaves produced were like those of the natural plants ($L/B = 1.85$) but they changed gradually until at the apex of the shoot the ratio L/B was 4.14. At this time the water contained only small traces of calcium, and hence it was probable that the changed leaf shape was chiefly associated with scarcity of calcium. A similar result was shown by the leaves from plants grown entirely in "excess potassium." The earliest leaves formed by these plants were broader and it was apparently only when the calcium in the solution and in the rhizome was used up, that the very elongate type of leaf was produced.

It will be realised from the above reference to the gradual change in the leaf form of plants grown in "excess potassium" that this fact accounts for their greater variability and consequently for the high probable error given in Table II for the L/B ratio of these plants. The variability of the different forms is summarised in the table below:

TABLE III

Number of leaves in different length/breadth classes from the given experimental conditions

L/B class	1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	4-4.5	4.5-5
1. Excess calcium	6	15	3	—	—	—	—	—
2. Calcium to roots only	1	12	1	—	—	—	—	—
3. Excess potassium	—	—	6	7	1	3	5	3
4. Calcium to shoots only	—	2	15	2	—	—	—	—
5. Natural leaves	6	16	6	3	—	—	—	—
6. Shive's solution (1/10)	—	4	3	3	3	4	1	—

The natural leaves were those from the parent plants and from a calcareous habitat. Their variation curve would clearly be similar to the curves for the experimental plants surrounded by excess calcium or rooting in excess calcium. The broad leaved form of *P. perfoliatus*

is therefore clearly a plant of calcareous soils, the character of the water being relatively unimportant. On the other hand, the narrow leaved form is produced more rapidly in excess potassium than it is in dilute Shive's solutions alone, and we are clearly justified in the assumption that excess potassium "antagonises" to some extent the effect of calcium when the latter is present in small quantities only. It is, in fact, only by the excess potassium treatment that large numbers of extremely narrow leaves are produced. It is probable, by analogy with permeability results, that other monovalent ions (e.g. sodium) would behave in the same way as potassium, and that other bivalent ions might produce a similar effect to calcium.

Summarising the experimental results, these are clear grounds for regarding the extreme variability of leaf form in *P. perfoliatus* as being due to the variations under natural conditions of (1) the light intensity and duration, (2) the calcium content of the soil, (3) the ratio of potassium (and monovalent ions generally?) to calcium in the soil if little calcium is present. There are grounds for supposing that other submerged species of *Potamogeton* show similar but less extreme types of leaf variation, particularly the closely allied *P. praelongus*.

An attempt has been made to estimate the effect of the experimental treatments upon the meristematic tissues, the comparison being confined to the details of plants grown wholly in the solutions with excess calcium or excess potassium (Nos. 1 and 3, Table II). The leaves of plants grown in excess calcium solutions show a much larger development of *minor* veins than do those from excess potassium solutions, although the *main* vein systems are very similar in the different leaves. The main vein system appears to be laid down and completed at a comparatively early stage, while the development of subordinate veins continues much later. It is difficult to compare accurately the young leaf initials. The very young leaves from excess potassium solutions are, however, both smaller and narrower than those from excess calcium. From the final sizes of the leaves and the constituent palisade cells (see below) it is estimated that the broader type of leaf contains approximately one-quarter more cells in length and twice as many in breadth as in the leaves from solutions containing excess potassium. Excess of calcium thus causes the leaf initial to produce about two and a half times as many cells as does a similar initial developed when potassium was in excess. Relatively, therefore, calcium must increase either the *rate* or else the *duration* of cell division to this extent.

In addition to this effect on cell division the experimental treatments produce well-marked effects on cell elongation. These are most easily compared by measuring the sizes of typical leaf cells.

TABLE IV

Average dimensions (μ) in cells of leaves of different origins

Source of leaf	Upper epidermis		Palisade cells (diameter)
	Length	Breadth	
1. Excess potassium	66.2	29.7	25.8
2. Excess calcium	28.0	27.5	20.0
3. Non-calcareous soil	18.8	27.2	—
4. Calcareous soil	10.5	20.6	—

(Nos. 1 and 2 are experimental—3 and 4 are natural plants.)

This table shows quite definitely that the cells are larger in the leaves when excess potassium is abundant, and since these differences in size cannot be detected at earlier stages in the development of the leaves it seems probable that they are due to modifications in the degree of cell extension produced by the experimental treatment. Two main factors at least are involved in determining the degree of extension of a cell, viz. the pressure exerted against the wall and the extensibility of the wall itself. Now potassium salts tend to increase the swelling of proteins more than calcium and we might perhaps assume by analogy that the cells in excess potassium are capable of exerting a somewhat greater pressure on their walls than those rich in calcium. There is, however, no evidence to show that these effects are capable of affecting the pressure on the cell wall to such an extent as to increase the size of the cell by two or three times as would be required to explain the results in Table IV. It is more usual, indeed, to regard the pressure against the wall as being due to the osmotic pressure of the cell sap and not derived to any material extent from the swelling of proteins present. If this were the case, then, the differences in cell size would probably have to be sought chiefly in differences in the extensibility of the wall produced by excess potassium or calcium. In this direction there is quite clear evidence that excess of potassium makes plant cell walls more gelatinous and extensible, while excess of calcium makes them harder and more resistant (Hansteen-Cranner(3)). This effect is due to the greater solubilities of the potassium soaps formed by the fatty constituents of the walls, and the consequent lack of rigidity in the wall is largely due to the absence of the insoluble calcium salts of these fatty materials. It may, therefore, be taken as proved that much, if not

all, of the increased size of the cells of leaves from solutions rich in potassium is due to the greater extensibility of the walls.

The cells of the stem internodes are also much longer when the plants are grown in "excess potassium" than when they develop in "excess calcium"—the relative proportions being about 5 : 2. This fact is associated with definite evidence that the composition of the outermost wall is different, for the cuticle is much more heavily cutinised (and approximately twice as thick) in stems from solutions rich in calcium (cf. also Lee and Priestley (4))—the calcium apparently assisting the deposition of the fatty materials to form the cuticle. No evidence of this sort is available for the leaf, chiefly because the cell walls are so thin that their examination is difficult.

It is possible that this wall effect may throw light on the fact that the leaf initials are larger when calcium is in excess. Hansteen-Cranner's results (3) indicate that water is evaporated more quickly from wall material soaked in calcium salts than from similar material soaked in potassium salts, and this may be because the material is more permeable in the presence of calcium—the fats being precipitated. Assuming this explanation to be correct, then it would obviously mean that a meristematic region would be more permeable to the diffusion of nutrient substances if calcium were abundant and we might then expect to get a greater rate of growth in the presence of excess of calcium. We have then the facts that in our experiments calcium accelerated cell division and retarded cell elongation and we have also at least one basis on which these facts can be explained.

A few words are necessary as to the effects produced by low light intensity. These, as pointed out previously, are similar to those associated with excess potassium but much less extreme. There is, however, the same reduction in the width of the leaf initials and the same elongation of the epidermal and internodal cells. On the whole, these characters may be, perhaps, attributed to partial etiolation. It is, however, possible that they may result from a scarcity of assimilates. This might cut down the rate of cell division and at the same time slow down the rate at which the walls were laid down, hence allowing greater elongation of the cells.

If we attempt to summarise these structural features, due to calcium or light, in their bearing on the problems of leaf form, then it must be concluded that the cell wall effect, while it modifies the final result by affecting the sizes of the cells, is chiefly of importance in suggesting that the growth rate may be associated with chemical and physical differences in the composition of the cell wall. These

are assumed to operate by altering the rate at which nutrient materials enter the meristems, a suggestion on which much further evidence is required. It is clear, however, that the main factor determining the shape of the leaf is the rate of growth of the leaf initial. This would appear to be determined under natural conditions chiefly by the calcium content of the soil and, to a less extent, by the light conditions. But doubtless other factors which affect the rate of growth, temperature for example, might also prove important.

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THE REPRODUCTIVE DIFFERENTIATION OF COLONIES IN CHLAMYDOMONADALES

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ALL the cells of the colony of *Pandorina* generally take part in reproduction, whether asexual or sexual. The writer has, however, recorded (1) colonies in which one to four of the cells remained sterile, were differentiated in staining reaction and showed signs of degeneration when division was complete in the other cells. It was stated that in one collection some forty colonies in the reproductive condition were examined. Of these only fifteen¹ were absolutely normal. The remaining colonies all contained special small cells, which were probably completely sterile.

¹ This number was misprinted as "fifty" in the original article, although corrected to "fifteen" in reprints.

The following supplementary information may be added to that given in the previous publication(1). It was argued that the forms described above are examples of a general tendency towards a sterilisation parallel to that which has resulted in the development of the *Pleodorina* and *Volvox* types of colony. The following additional facts show that there is a special tendency to this form of differentiation in abnormal specimens of *Pandorina* and other allied forms, which are generally described as having colonies in which every cell is fertile. Prior to the publication mentioned Powers had briefly referred to forms similar to, although not identical with, those recorded by the present writer. In a paper on new forms of *Volvox* (5) he says: "*Pandorina* has shown a wholly unwarranted method of asexual reproduction, a method in which single cells, instead of all the cells, gave rise to new colonies, suggesting at least the parthenogenesis of *Volvox*." It appears that these forms chiefly differ from the present writer's in the fact that the reproductive cells were isolated amongst the sterile cells. Presumably the latter were more abundant than in those found by the present writer, where the one to four sterile cells usually formed a small group at one end.

As far as its morphology is concerned, the latter form bears precisely the same relation to *Pandorina morum* Bory that *Pleodorina illinoisensis* Kofoid does to *Eudorina elegans* Ehrenb. Now W. and G. S. West (7) have actually recorded and figured variants of *Eudorina elegans* Ehrenb. which bear the same relation to the type forms as Power's variants of *Pandorina*, described above, do to typical *Pandorina morum* Bory. Unlike *Pleodorina illinoisensis* Kofoid, these forms have an indiscriminate arrangement of large and small cells, "one size of cell not being restricted to a definite part of the coenobium" (7).

The tendency to produce sterile cells also exists in *Stephanosphaera phlvialis* Cohn. for colonies of this species are recorded (4) in which not all the cells take part in reproduction.

Since the presence of a differentiated somatic area in the colonies has been used as a distinctive systematic character among colonial Chlamydomonads (*Pleodorina*, *Volvox*), the question of the taxonomic status of all the above described variants now arises. A comprehensive account of the characters of a species, such as is necessary to determine its systematic position, must also include the facts of individual variation. The more aberrant variants of a species often serve to link it up with other types. Thus *Pleodorina illinoisensis* Kofoid is frequently found associated with forms apparently identical

with *Eudorina elegans* Ehrenb. Forms transitional between typical individuals of these two species exist (8). It has been assumed that there is an ontogenetic connection between them (8).

In the previous paper *Pleodorina illinoisensis* Kofoid was regarded as a variety of *Eudorina elegans* Ehrenb.¹ The existence of variant colonies of *Pleodorina* resembling in all respects those of *Eudorina* is not proof of the identity of these genera, since in the life-cycle of a species forms resembling other species may exist (2). However, the variants of *Pleodorina* recorded by West (8), Grove (3) and others at least show clearly that an affinity exists. The abnormal variants of *Pandorina*, *Eudorina* and *Stephanosphaera* discussed in the preceding paragraphs may possibly belong to genetic strains distinct from the normal type. At present there is not sufficient evidence for describing them as distinct species. The following system of nomenclature may, however, be of use in the cataloguing of the variations in respect to somatic cells. *Pandorina*, *Eudorina*, *Stephanosphaera* and many other colonial forms belonging to this and other cycles of affinity generally exhibit colonies in which every cell takes part in division. Such colonies may be said to belong to type α . The division of the various cells of the colony may be either simultaneous or successive. The latter condition leads over to other types of colonies in which certain cells, corresponding with those which divide later in the former type, are definitely somatic, for they fail to divide and eventually degenerate. Amongst the latter there are two distinct types of differentiation. In the one, which is represented by the genus *Pleodorina*, the somatic cells are arranged in a definite area in one part of the colony. This may be spoken of as type β . In the other, which is chiefly represented by the genus *Volvox*, the somatic cells are spread amongst the reproductive cells throughout the whole colony. This may be called type γ .

The colonial forms referred to in this article can then be classified as follows:

A. *Cells spherical*

Eudorina α = *Eudorina* Ehrenb.

Eudorina β = *Pleodorina* Shaw.

Eudorina γ = *Besseyosphaera* Shaw.

The only described species of this genus is *B. Powersi* Shaw (6), a type chiefly distinguished from *Pleodorina* in having the asexual reproductive cells scattered amongst the vegetative cells. The colony

¹ The statement was followed by a reference number (8, etc) which should be corrected to (9, etc) and refers to Grove's paper.

is large, consisting of about 1000 cells, but differs from *Volvox* in having no protoplasmic connections between the cells.

The forms described by West(7) must also be included in *Eudorina* γ . They differ from *Besseyosphaera Powersi* Shaw in their smaller size, and may be termed *Eudorina* γ *Westii* or *Besseyosphaera Westii* nom. nov., according to whether the type is regarded as a variant or as a definite species.

B. Cells pyriform

Pandorina α = *Pandorina* Bory.

Pandorina β = forms described by the writer (1).

Pandorina γ = forms mentioned by Powers (5).

C. Cells amœbiform

Stephanosphaera α = *Stephanosphaera* Cohn (typical form).

Stephanosphaera β = forms mentioned by Hieronymus (4).

It must be understood that the above is merely a summary of colony forms on the basis of their differentiation into vegetative and reproductive areas. It is therefore not intended to express a system of phyletic relationships, for which a review of the sex differentiation and many other characters would have to be included. It is intended to supplement, rather than to replace, the existing system of generic names, for it is probable that several, if not all of the forms referred to, are genetically distinct. The facts discussed in this article, however, are insufficient data on which to base a revised taxonomic nomenclature.

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THE GEOGRAPHICAL DISTRIBUTION OF THE GENUS *SPHAERANTHUS*

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(With 6 figures in the text)

IN a previous paper¹ on the genus *Sphaeranthus* Vaill. (Compositae) I was mainly dealing with its classification and possible phylogenetic evolution.

I now take the opportunity of pointing out some outstanding features in its geographical distribution, and bring forward further evidence regarding the undoubted great affinity between certain East African and Indian species.

The genus *Sphaeranthus* is chiefly confined to the tropics of the old world, its northern and southern limits corresponding almost with the tropic of Cancer and the tropic of Capricorn respectively. Only the four most widely spread and best known species slightly extend beyond the northern boundary: *S. suaveolens* DC. (*Oocephalae*), and *S. africanus* Linn., *S. indicus* Linn., *S. senegalensis* DC. (all three *Sphaerocephalae*). In addition, *S. strobiliferus* Boiss. et Noë (*Oocephalae*) seems to be endemic in Persia as specimens are as yet only recorded from that region. As to the southern limit given above, *S. peduncularis* DC. is only known by specimens from Natal, and *S. incisus* Robyns is mainly distributed in the Transvaal and Swaziland, but also occurs as an outlier in tropical South Angola. These two closely allied South African species belonging to the section *Oocephalae*, and differing mainly by the deeply incised wings of the branches and the shorter peduncles of *S. incisus*, have also a similar geographical distribution. The western limit of the genus extends as far as the Cape Verde Peninsula, but only one species, *S. senegalensis*, has nowadays been met with in this far West African region, no other species spreading more westward than Northern Nigeria. The eastern limit of distribution is to be found in the Philippines (Luzon) and North Australia, but again only two species are recorded from these regions, and all the others are confined to the Asiatic Continent.

¹ W. Robyns, "Revision of the genus *Sphaeranthus*" in *Kew Bull.* No. 5, pp. 177-199, 1924.

Considering separately each of the four sections of the genus established in my paper already referred to, I notice at once the large distribution of the sections *Oocephalae* (subgen. *Pseudo-sphaeranthus*) and *Sphaerocephalae* (subgen. *Eusphaeranthus*) compared with the small extent of the sections *Platycephalae* (subgen. *Pseudosphaeranthus*) and *Cylindrocephalae* (subgen. *Eusphaeranthus*). The species of the latter two sections are almost entirely limited to the tropical East African continent, except one species which is endemic in Madagascar, whilst the species belonging to the former two sections extend both into tropical and subtropical Africa.

The species of the section *Oocephalae* are spread all over East Africa and in Persia, South India and Burma, but each of them is confined to only one of these continents, no common species occurring amongst them. Yet the great affinity between the East African *S. cyathuloides* O. Hoffm., and the South Indian *S. amaranthoides* Burm., is so striking that one may be entitled to consider these two species as equivalent in the two areas. The species belonging to the two small sections *Platycephalae* and *Cylindrocephalae* show also quite a local distribution. Hence the only species we might consider as endemic occur only in these three sections. Amongst the most evident endemics the following are especially to be quoted: *S. strobiliferus* Boiss. et Noë, a representative species from Persia; *S. peguensis* C. B. Clarke, from Burma; *S. peduncularis* DC., from Natal; *S. cotuloides* DC. and *S. madagascariensis* Robyns, from Madagascar; *S. sphenocleoides* Oliv. et Hiern, from Zanzibar. Most of these endemic species are so strikingly different from the others that they are at once recognisable amongst them. This seems to be especially the case of the Persian and Madagascar species.

The last section, the *Sphaerocephalae*, shows by far the widest distribution; out of the nine species included in this group, three have large areas of distribution and are common both to Africa and to Asia, the remaining ones being only African.

S. africanus is mainly represented in Asia, the Malay Archipelago, the Philippines and North Australia. It also occurs in Madagascar, but curiously enough no specimens have yet been recorded from Africa proper. However, *S. sphenocleoides* from Zanzibar, which is evidently very similar to *S. africanus* and which may be only a very distinct form of the latter, can perhaps be looked upon as an African link of it. *S. indicus* occurs in East Africa, Madagascar, Asia and Australia; it is well worth noticing that in Asia it extends into South Western China. But the most remarkable and uncommon

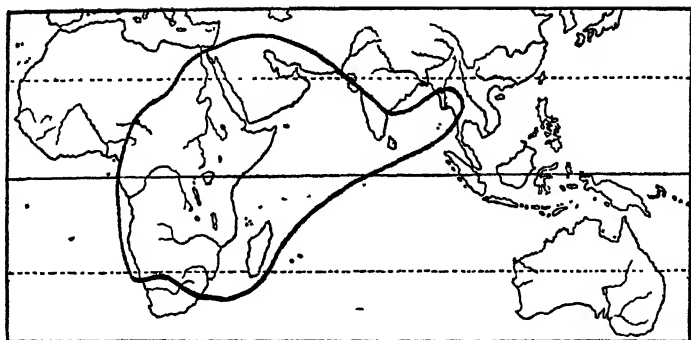


Fig. 1. Range of section *Oocephalae* of subgenus *Pseudosphaeranthus*, including the species regarded as the most primitive of the genus *Sphaeranthus*

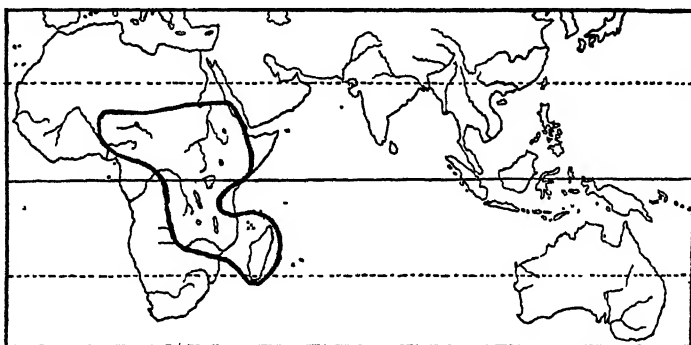


Fig. 2. Range of section *Platycephalae* of subgenus *Pseudosphaeranthus*

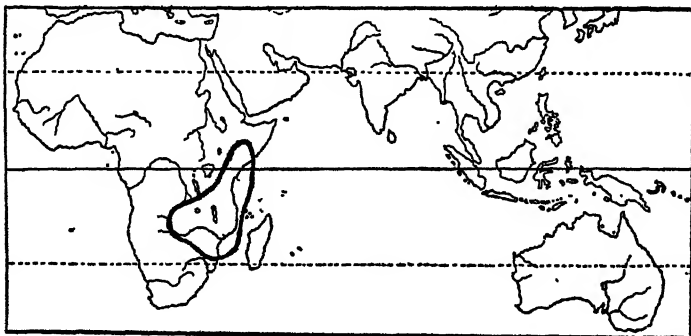


Fig. 3. Range of section *Cylindrocephalae* of subgenus *Eusphaeranthus*, perhaps the most ancient group of this subgenus

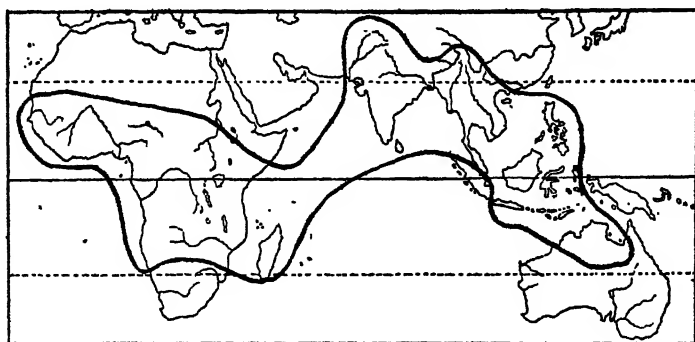


Fig. 4. Range of section *Sphaerocephalae* of subgenus *Eusphaeranthus*, including the species with the widest distribution and regarded as the more recently evolved

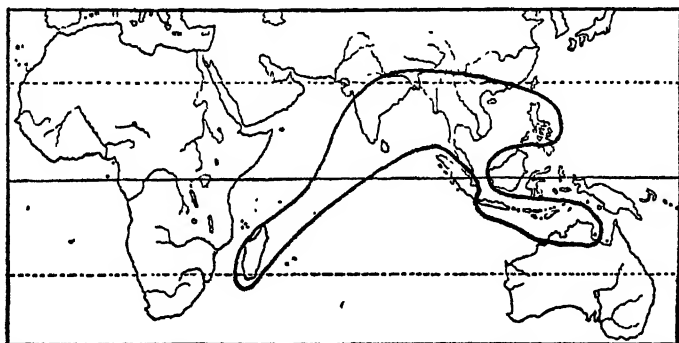


Fig. 5. Range of *Sphaeranthus africanus* L. of the section *Sphaerocephalae* of subgenus *Eusphaeranthus*

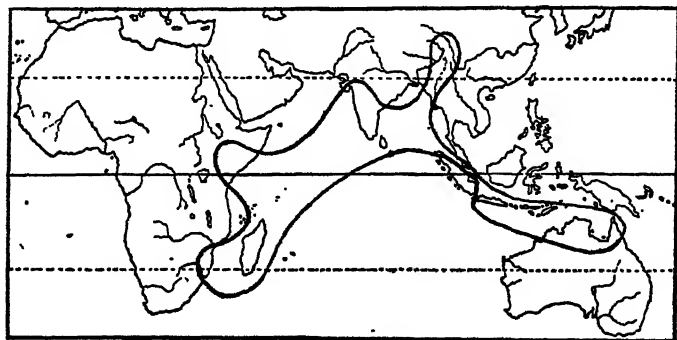


Fig. 6. Range of *Sphaeranthus indicus* L. of the section *Sphaerocephalae* of subgenus *Eusphaeranthus*

distribution doubtless belongs to *S. senegalensis*, which is recorded from western tropical Africa (Cape Verde Peninsula, Senegambia, N. Nigeria), N.W. Rhodesia, N.W. India and further India. Either this species is very ancient in the group or it is specially adapted to a wide distribution and more or less of a weed.

The main features, as briefly dealt with above, strongly support the view that the central area of distribution of the species of the genus *Sphaeranthus* is to be found in East Africa and India, suggesting without doubt a close geographical connection or even continuity between these two regions in the past. The affinity of the rich Madagascar flora and the Indian flora is again very evident. It is also significant that the section *Oocephalae*, which may be looked upon as the less specialised group of the genus, is represented actually by the greatest number of species, of which sixteen are African and only four Asiatic. This, moreover, emphasizes the view that the primitive headquarters of development of *Sphaeranthus* is likely to be placed on the East African continent, especially as the two most primitive species, *S. cyathuloides* and *S. Johnstonii* Robyns, are almost entirely African. Furthermore, the group *Platycephalae*, which we consider as being merely a greater specialisation of the *Oocephalae*, and the group *Cylindrocephalae*, which is likely to be the most primitive section of the second line of development appearing in the subgenus *Eusphaeranthus*, are also only confined to Africa. Finally, the most recently evolved and highly specialised group of the *Sphaerocephalae* appears to be the more successful as it is the most widely spread, containing the most largely distributed individual species.

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ENGLISH AND GERMAN BOTANY IN THE MIDDLE AND TOWARDS THE END OF LAST CENTURY

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AT the middle of the nineteenth century British Botany was marked by strange contrasts. It witnessed the production of comprehensive works, often of fundamental importance, by its seniors, such as the *Species Filicum* of Sir W. Hooker, the *Antarctic Flora* by his son Joseph, together with the *Genera Plantarum* of Bentham and Hooker, the *Flora of British India*, and as text-books *The Vegetable Kingdom* of Lindley, and Balfour's *Classbook*. But overshadowing all was the *Origin of Species*. On the other hand, for several decades, it failed entirely to produce the students who were to take the places of these veteran authors, while they themselves were for the most part free from didactic duties. After the interesting tenure of Daubeney, the Oxford chair fell into the hands of Lawson. At Cambridge Babington taught dry systematic, and very little of it. Scotland was better placed, with Hutton Balfour in Edinburgh, who initiated practical demonstration in the laboratory. Glasgow was didactically somnolescent under Walker-Arnott, but Dickie was active and alive in Aberdeen. The destinies of Dublin were guided by the great algologist, Harvey. Nowhere in England did the teaching extend beyond the set lecture, though in Scotland this was amplified in the most practical way by excursions in the field, often of considerable extent and duration. These were established by Sir William Hooker in Glasgow, and the field work became a marked feature under the régime of Hutton Balfour in Edinburgh. Collecting, classifying, and recording were the order of the day, while anatomy, physiology, and the study of the complete life-cycle, though not actually neglected, were given a minor place in the University curricula.

It seems strange now to look back upon, and note the deadness of the educational methods of that time of extreme brilliancy in individual production—to read the *Life and Letters* of Darwin, of Hooker and of Huxley, which reveal the intense personal activity of those giants of the middle decades: and then to turn to Oxford and Cambridge, and realise their sterility at the very time when the *Origin of Species* was published. The young foundations of University College, London, and of Owens College, Manchester, were not able yet to adjust the balance, while the Scottish Universities devoted their attention almost entirely to the botanical requirements of their medical schools. For more than ten years after the publication of the *Origin of Species* this torpor lasted. But it was one of the protagonists of Evolution who finally dispelled it:—Huxley, the centenary of whose birth occurs this year.

Before we can properly estimate or understand the nature of that re-awakening of anatomical and physiological research in this, the very country of their birth, a glance must be cast upon the position of similar studies on the continent about the middle of the last century. The arid detail of the phytotomists had received a shock from the hand of Schleiden. That valley of dry bones was stirred into life by his dictum that to understand form and structure we must seek their elucidation in development. Thus a real morphology was born; that word initiated by Goethe now first received an intelligible meaning, and a new branch of science emerged from a nebula of poetic mysticism. The road, already paved by the structural observations of Von Mohl and by the knowledge of the plant cell as revealed by Schwann, Robert Brown, Naegeli and others, soon became an open highway for the illuminating researches of Hofmeister. This is not the place to evaluate the work of that great man: last year the centenary of his birth was celebrated by a volume from the pen of his sole remaining pupil, Professor Von Goebel, of which an English version is shortly to be published by the Ray Society.

While we admire and appreciate to the full the amazing results attained by Hofmeister, we should not forget that work of the highest significance was being done during the same period in France. Relating as it did to the Algae, it might not at the time appear to bear the same constructive value as that of Hofmeister. But as the search for origins probes ever further downwards, the results of Bornet and Thuret are now being evaluated afresh. By a happy collocation of wealth, scientific acumen, and artistic skill, Thuret,

Bornet, and Riocreux produced in their *Études Phycologiques* in 1878 a folio of the most sumptuous form, embodying researches on Algae conducted chiefly at Antibes, where Thuret had a coastal villa. These basic researches on marine Algae dated from 1846 onwards. Thus they ran parallel in time and in spirit with those of Hofmeister on the Archegoniatae. It has taken three quarters of a century to interpret their results in terms of the nuclear cycle. But in the demonstrations of Bornet and Thuret we see many of the basic facts upon which rests securely the present knowledge of the Algae. The publication of their collected work took place the year after I had graduated at Cambridge. I have sometimes wondered what would have been the result if that splendid volume had fallen into my hands at the very time when I was busily scanning the shelves of the University Library for literature on the Algae. I might have visited Antibes instead of Würzburg or Strassburg, and have become an algologist, as indeed I was once inclined to be.

We now see clearly enough how in their torpid blindness the British Universities had missed the significance of those great continental changes of which the works of Hofmeister, and of Bornet and Thuret were the sign. While Babington was splitting analytically the varieties of *Rubus*, Hofmeister was revealing constructively the alternating cycle in all land vegetation. Cambridge was fiddling with details, while Leipzig and Heidelberg were burning with a new synthetic flame. But the time was near for a still brighter vision. Within a few years of Hofmeister's *Vergleichende Untersuchungen* (1851) came the *Origin of Species* (1859). It then appeared as though a theory of evolution had merely to adopt the results already demonstrated by him. After events so stirring we need not be surprised that a pause should follow. It took time for men to realise the bearings of the new views, and still more to convert them into action. Botanically little change appeared immediately in Britain: but within a decade an event happened in Germany which was to produce far-reaching results. It was the publication of the *Text-book* of Sachs.

That work was written with consummate judgment, and illustrated with all the skill of a trained draftsman, who was also a keen observer. Sachs was, I believe, the son of a wood-cut artist, and had been himself trained as such. But beyond his keen vision and artistic touch, he possessed also a philosophical outlook combined with keen receptivity. These faculties made him the best possible exponent of the results of those, such as Hofmeister, whose powers

of exposition were less than his own. Little wonder that the *Text book*, which embodied not only their results but also a vast extent of Sachs' own observations, morphological and physiological, had a phenomenal success. The first English edition published in 1875 was based upon the third and fourth German editions, and it fell into my hands at once on its appearance. It came as a revelation to the group of enthusiasts beginning to gather round Vines at Cambridge, and supplied the text upon which much of our work was based. We felt then that we were daily seeing things not, it was true, new to science, but at least observed for the first time in Britain. Some of us, however, naturally looked further to the living source: and when Vines went to Würzburg in 1877, though still an undergraduate I joined him for some weeks in the summer, to sit at the feet of Sachs himself.

I need not describe my experiences there, partly because Vines has already written of that time (24, p. 2): partly because my ignorance of German prevented my taking the fullest advantage of the opportunities. What I gained was personal contact with the professor, who spoke English for my benefit. He taught me the current Hofmeisterian methods, but he also led me into the lines of exact delineation. I remember his saying cryptically, pencil in hand, that "Every drawing conveys a view." Unconsciously he thus pointed the difference between his own method and the modern photographic illustrations, which so often cover the inefficiency, or it may be the laziness, of current authors. For while Sachs's figures are the most explicit ever published, modern photographs often fail adequately to represent either the object, or the view of the author. Sachs was very dexterous in manipulation, and I remember his practising the Hofmeisterian feat of cutting a single fresh and unembedded ovule into sections between the finger and thumb, and spreading out the sections on a slide. We embedded sometimes in gum, and I remember cutting in this way by hand a beautiful section of a pollen grain of *Malva*. I did not realise then, as I do now, that the work I was set to do was a *mélange* of Hofmeister, Pringsheim, and of Sachs himself. I was frequently the only worker in the public laboratory, a fact that caused me some surprise: though I have since learned that it was not an uncommon thing for German laboratories to be almost empty of German students.

Some years before this visit to Würzburg events in Britain had been moving towards that revival of laboratory study which culminated in the seventies and eighties. Up to the middle of the

nineteenth century authoritative statement by the teacher rather than personal observation was the source of knowledge for the ordinary student. It is undoubtedly to Huxley that we owe the initiation of that systematic laboratory training which has now become general. He laid special stress upon personal observation at first hand as the leading feature of biological study, even for elementary students. He did not abolish the lecture room, but he linked it with the laboratory, so that the student duly primed with a vivid description of what others had seen should pass to the laboratory to see, confirm, or criticise for himself. Those who have grown up under this newer method will with difficulty realise the revulsion thus brought about. Its effect was at a single stroke to convert each student into a potential investigator. On the other hand, the new method would react inevitably upon the teacher. Knowing that any or all of his students might form an independent estimate of the matter in hand, he must not only be secure in fact, but also be ready for discussion. Every laboratory class became at once a board of oral examination for the demonstrating staff.

Huxley's opportunity came in 1871, as a necessary consequence of the inclusion of elementary science under the Education Act of 1870. If science is to form part of the curriculum of the schools of the country, the teachers must themselves be taught: and South Kensington was the centre, and Huxley became Dean of the Science School. The first course for schoolmasters took place in 1871, and was a more or less makeshift affair. The first in the new laboratory was in 1872. We may imagine what kind of courses they would be under the direct management of Huxley himself, assisted by Burdon Sanderson, Martin, Thiselton-Dyer, and Ray Lankester. The course for beginners was soon crystallised into the well-known volume on *Elementary Biology* by Huxley and Martin. Thus the method became stereotyped. Where the book fell into less expert hands, and its spirit filtered through less potent minds, the results would necessarily be less satisfactory: but that fact does not discount the excellence of the method. Very soon more detailed courses were devised, respectively on animals and on plants separately. Those on plants were conducted by Thiselton-Dyer, the first being in 1873. I demonstrated to some of the earlier of these, with Vines, Marshall Ward, and MacNab as colleagues.

I felt after graduation at Cambridge that the short visit to Würzburg in 1877 was an insufficient introduction to German methods, and in the summer of 1879 I started, with my brother as

a companion, to Strassburg, where we found Vines already at work. Our object was to attend the new German University founded there after the war of 1870. Particular care had been taken in the selection of the professors, and a natural choice fell on De Bary, not only from the fact of his outstanding scientific claims, but also of his Alsatian origin. I spent there a whole academic year, receiving from the Professor the utmost kindness and help. Solms-Laubach was there as Privat Docent, working at Gymnosperms, with a separate room, and we seldom saw him. Klebs was Assistant, constantly present and conversational. But the direction came personally from De Bary, whose room opened out on the general laboratory, and he was always accessible. The buildings were those of the old French "Académie," low-roofed, and ill-lighted: while the Botanic Garden was little more than a closed yard, within the old French fortifications then being demolished. The ring of new German earth-works, moats, and isolated forts was far advanced in construction, but the new Institute and Garden outside the old line still existed only as a scheme. The city had recovered from the bombardment, though a few marks were still visible upon the cathedral, which stood the siege virtually uninjured. The military were in evidence everywhere, and particularly at the theatre. Field Marshal von Mannteufel might be seen daily, walking in the garden of the "General-Commando."

The laboratory contained half a dozen nationalities among its regular workers: but I only remember one ordinary German student, Stephan, a "freiwilliger," who used to come in occasionally in Uhlan's uniform. There was a Belgian (Errera), afterwards Professor in Brussels; a Swiss; two Italians (Pirotta, now Professor in Rome, and Matirollo, now Professor in Turin); an Alsatian (A. F. W. Schimper); while Vines and I represented Britain. There was also a fresh memory of the American, Farlow, and of his work on apogamous Ferns. An intermittent stream of visitors passed through, such as Zacharias, Stahl (who was an Alsatian from Kehl), Victor Meyer, F. Darwin and Marshall Ward, Elfving, etc. Strassburg was then no doubt the vital centre of academic botany in Germany. For De Bary was in 1879-80 at the full plenitude of his powers. He had a slight figure, and a rather drawn invalidish face, which was explained by his frequent attacks of neuralgic pain. His movements were quick, as his mind was alert, so that his advice and help in the laboratory proved pointed and stimulating. I still remember the sound of his regular morning question, "Na! Wie geht's?" or "Was

giebt's neues?" As a lecturer his effect depended on his material rather than on his delivery. During the winter he gave advanced lectures twice a week on anatomy, at 2-4 p.m.—less the "akademisches Viertel." We were all there at 2.15, when he would slip in behind the small lecture table, and for a minute or two walk up and down like a caged lion, muttering inaudibly to himself. Then he would suddenly face round to us, and begin the actual lecture. Personally I was firmly held by it during the whole of the long sitting. But there was little accessory help from forensic art, or pictorial illustration: his success depended upon the actual substance of the discourse. The time to catch him for personal conversation was when he left for "Mittagessen." He would suddenly open the door of the laboratory, and ejaculate sharply, "Wer kommt mit?" Then you must spring up at once and hurry down stairs, or you would miss him, for he was off like a rabbit. When you did catch him, you would enjoy a delightful breezy, though rather breathless walk with him across Strassburg, till he vanished into the Blauwolkengasse.

The winter of 1879-80 was one of the coldest. The thermometer was frequently below zero Fahr., and I well remember the cheerful tones of the ex-Uhlan, Lanfried, the laboratory attendant, when about 9.30 a.m., he would enter and announce, "Vierzehn Grad, meine Herren." That meant 14 degrees below freezing *Réaumur*, and those who will may calculate its equivalent Fahr. The wheels of the carriages creaked and squeaked upon the hard frozen snow. The frost brought skating on the floods towards Kehl: and I have a vivid memory of Klebs with obvious joy, cutting great sweeps on the outside edge, his spare leg cocked up in an ungainly attitude accentuated by his short green-braided Jaeger jacket, with two green buttons between the shoulders, and two green pom-poms hanging from his Tyrolese hat!

Throughout the academic year De Bary put me individually through a capital course of general anatomy, based upon his own great comparative work. I remember in particular a spirited hunt after the protoxylem in Tree-Fern stems, where it is virtually non-existent. I also carried out a minor enquiry into the growth in thickness of the cell wall in the sclerotic endodermis of the root in *Dracaena*, in which I practically demonstrated a process of apposition, then a burning question. But though I still have the drawings they were never published. In the spring of 1880 I worked on Fungi, and particularly on the Rusts. The intervals of such work were

devoted to a definite research, which lasted throughout my stay in Strassburg. In the summer of 1879 I brought out with me young sporelings of *Fucus* (as I thought), collected at Flamborough Head the year before: and I set to work on them to establish the origin of the apical growth of the adult. De Bary was interested and sympathetic. He suggested mounting my sections in glacial acetic acid, a method used by Rostafinsky. I remember still its effect on my eyes in the hot weather. The research came to nothing, for I soon found that my material was a mixture of various Fucoids. So I passed on to the development of the conceptacle in *Fucus*: this resulted in my first paper, which was published in the *Q.J.M.S.* 20. According to our English custom, I made no secret of the subject of my work. It was part of the common talk of the laboratory. During the whole of that academic year I sat side by side with A. F. W. Schimper. I never knew what he was at work upon. I never saw his drawings: whenever he consulted De Bary it was in the private room, not in the general laboratory. It was only when his paper was published in the *Botanische Zeitung*, and he sent me a copy of it, that I learned that his work was upon plastids. Such precautions may have been necessary elsewhere, but they are foreign to our British laboratories, where we habitually make common stock of our ideas and our efforts.

In the spring of 1880 I returned home, and within a few months found myself engaged as assistant to Professor Daniel Oliver, and in full charge of the practical classes then for the first time established at University College, London. Looking back upon this period of preparation, there is no doubt that it was necessary at the time that some of us should become personally acquainted with German laboratories and methods. Going to foreign schools was the readiest way of making up that backwardness which had resulted from the academic apathy of a generation of the teaching botanists of Britain, and from their failure to keep pace with those advances in observation and laboratory technique which had grown up on the continent. A broad outlook may perhaps help to explain how the difficulty itself arose. A great expansion of Imperial interests had taken place in the early Victorian time. The whole energy of Kew, of the British Museum, of Edinburgh and Glasgow, and in a minor degree of other centres, had been concentrated upon the floristic exploitation of the British Dependencies. India in particular had pressing claims. The cataloguing of the floras of these lands raised far-reaching questions of geographical distribution

which worked in readily with the nascent views as to descent. It was indeed the commanding interest of such matters which tended to drift attention away from the intensive study of the laboratory. Unfortunately even the most active professors of the time allowed their interest to be carried away by the set of the Imperial stream, though it ought to have been spread over the whole sphere of botanical science. This resulted in a lop-sided state, from which it needed time and a minor revolution to recover. That strange period still has its lesson for us to-day. At the present moment the tendency of British Botany is once more towards concentrated application to Imperial needs of another sort from those of the Victorian Age. Agriculture, Forestry, Plant-Pathology, and Plant-Breeding all rightly command the interest of young aspirants, and the State is beginning to offer the inducement of salary, attainable on rapid qualification. It is for those who direct the progress of the teaching of the science to take care that they shall not, by allowing too early specialisation along applied channels, send out specialists too quickly and imperfectly qualified. But the more grave risk is that they should allow the central institutions to lose again their hold upon the broad stream of pure science, as their predecessors did at the middle of the last century, by over-concentration upon special requirements. The Empire needs the progress of Botany at large, not only of one or another phase or aspect of it. The obligation rests upon the Heads of Departments to see that an even balance shall be maintained: so they will best prevent a period of lop-sidedness, and avoid all need for another revival like that of the seventies of the last century.

THE SECRETORY SYSTEM OF THE ROOTS OF THE COMPOSITAE

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(With Plate III and 3 figures in the text)

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I. INTRODUCTION

THE cortex of the majority of dicotyledonous roots is an extraordinarily uniform tissue with regard to its structure, and also to its developmental history. In its youngest stages just behind the meristem, it consists of several layers of more or less uniform parenchymatous cells showing no peculiar structural features. The piliferous layer and later the exodermis form a complete cylinder round the cortical cells within and, in the case of the exodermis, no intercellular spaces are present between the individual cells. Towards the inside, the cortex is separated from the vascular cylinder by the endodermis, a layer showing various peculiarities in structure which enable it to control, to a very large extent, the food supply to the cortex. The endodermis, like the piliferous layer, forms a complete cylinder, and the radial walls of the individual cells are characterised by the Casparian strip, which has been shown to consist principally of fatty substances of a varnish-like consistency (Priestley and North(8)). The endodermal cells contain living protoplasts which have been welded in with the Casparian strip during its deposition on the radial wall. The endodermis in its primary condition is permeable to water but all water soluble food substances entering from the soil must pass across the protoplasts in their passage from the soil to the vascular cylinder.

Later stages in root structure will show a change in the structure

of the endodermis. This will have reached now the secondary stage in which the radial and transverse walls of the endodermal cells will be covered with a suberin layer. The presence of such a layer alters the relations of all the cell layers outside to their food supply, more especially when an exodermis with fat impregnated walls has been formed on the outside of the root. At this stage, the cells of the cortex are dependent for their food supply on that contained in the vascular cylinder. All food to the cortical cells must of necessity pass across the endodermis. A suberin lamella such as that found on the endodermal layer has been shown to be impermeable to water and its solutes (Priestley and North) and consequently it forms a very effective barrier between the cells of the cortex and their food supply. As a necessary consequence of this fact, the cortex gradually dries up and finally disappears, leaving the endodermis for a time as the outermost layer of the root. Before the disappearance of the cortex, however, cell divisions are taking place within the endodermis to form cork. With the increase in growth of these cork cells, the endodermis is ruptured and the cork becomes the outermost protective layer of the root. Throughout this typical sequence of events, no new structures will be initiated in the cortex and all new developments take place within the endodermis. This characteristic can be correlated with the peculiarities of endodermal structure just described. Additional evidence in support of this statement has been obtained in this laboratory by Miss Lettice M. Woffenden in an extensive survey of the anatomy of roots in connection with root propagation. It was found that many lateral roots emerged surrounded by callus-like pads of tissue produced secondarily in the cortex even in roots having a secondary endodermis. These secondary growths were found to be associated with a break between the endodermis of the parent root and that of the lateral root. Such a break would thus enable the necessary food to pass out to the cortex in the region of the lateral root.

During work done in this laboratory on propagation by means of isolated pieces of root, several members of the Compositae came under observation. It was noticeable that, anatomically, these roots differed in several important respects from those of the typical dicotyledons just described. Several members of the family formed exogenous cork and exogenous buds, and appeared to retain their primary cortex for a much longer period in the life history of the root than was the case in the members of other families. The whole root was found to contain a large amount of fatty substances and

in particular the endodermis. When heated in Sudan III, the fats in this layer broke up very easily into minute globules, thus suggesting that the suberin lamella was saturated with an oily fat instead of forming the usual rigid strips, deposited on the walls of this layer of cells. Another well-known peculiarity of Composite roots is the presence of intercellular canals just beyond the endodermis. Also, in older roots, additional intercellular canals may be formed in the cortex, medullary rays and secondary phloem. More detailed investigation was begun in the light of recent work on the endodermis and also on account of the extraordinary supply of fat in that layer, which, it was thought, might throw new light on the formation of these canals. As a result of this investigation, new suggestions are now brought forward as to the origin and nature of the canal and its contents. Earlier work on the subject, which is reviewed in the next section, has either been based on a small amount of material, sometimes in a mature stage, or it has been done almost entirely from the point of view of the classification of the group when the descriptive study of the canals was of primary importance.

The result of this investigation has been to confirm the idea that the unusual cortical activities of Composite roots were closely bound up with the peculiarities of the endodermis briefly described. In Composite roots, the organic substances, which in typical dicotyledons are confined to the vascular cylinder, are finding their way into the cortex across the endodermis. This leakage is connected with the fatty nature of those organic substances and the unusual amount of fat in the endodermis which makes such a leakage possible. Whilst resins and ethereal oils may be amongst the substances secondarily formed in the canals, preliminary chemical studies make it clear that fatty substances form the most important part of the original secretion.

The present paper is practically confined to a re-examination of the cortical secretory system of these roots, especially the endodermal secretory canals. A short account, however, of bud formation in the cortex is included for the purposes of discussion and a more detailed account will appear when recent studies of vegetative propagation are published and with these studies the name of Miss Lettice M. Woffenden will be associated.

2. HISTORICAL

The first mention in botanical literature of a secretory system in Compositae, in addition to the latex vessels, is that of Julius Sachs

in 1859, who discovered the secretory canals in the stem of *Helianthus annuus*. He recognised that they were specialised intercellular spaces occurring opposite the phloem.

In 1862 Trécul distinguished between these secretory canals and latex vessels by the fact that the latter were limited by their own walls while the former were without any such layer. Trécul then thought that the one was derived from the other. Mueller in 1867 confirmed the fact that the secretory canals were intercellular spaces and that they were often bordered by four small cells which may or may not divide.

A considerable amount of work on this subject was done by Van Tieghem and Vuillemin between 1872 and 1886 (14, 15, 16). Van Tieghem showed that these secretory canals were of common occurrence throughout this group and he emphasised the fact that, in the case of roots, they were formed in close connection with the endodermis. Most of his work, however, dealt with the secretory apparatus of the stem. Vuillemin and Van Tieghem discussed the difference between the secretory canals of the stem and root in papers published in 1883 and 1884. They held that the outstanding difference between the two was, that in the stem the canals were surrounded by specialised cells, while in the case of the root, they were considered to be merely intercellular spaces formed in close connection with the endodermis. In Van Tieghem's paper of 1883, he distinguished three kinds of secreting apparatus: (1) oil canals, (2) laticiferous canals, (3) resin canals. The difference between (1) and (3) evidently, is based on the supposed difference in chemical content of the two kinds of canal.

In 1885 Triebel (12) published a detailed description of the development of the oil canals in the roots of a few members of this family. He found that the formation of the canals was always preceded by the tangential division of the endodermal cells opposite the phloem—the canals beginning as minute intercellular spaces at the junction of the radial and newly formed tangential walls. These canals became filled with drops of almost colourless oil at a very early stage. The cells surrounding the oil channels had thinner walls and were much shorter than those of the cortex proper, they were meristematic in appearance, being filled with dense protoplasm. He considered that these cells probably played an important part in the secretion of the oil into the canal. The oil was termed an ethereal oil, a conclusion based on the following facts: (1) that most of the roots examined had a characteristic smell, (2) that the oil was soluble in alcohol, and (3) that it stained with alkanet.

The next important piece of research and probably the most extensive work done on the subject is that of Col(2), whose results were published in a series of papers between 1899 and 1904. He, however, studied the secretory apparatus mainly from the point of view of the classification of the group. He also distinguishes three classes of secreting apparatus, which however differ from those of Van Tieghem: (1) anastomosing latex vessels, (2) secretory canals, (3) isolated cells which secrete latex. Van Tieghem's oil canals and resin canals are both included under the general term secretory canals. Col recognised two forms of secretory canals: (a) canals proper, which are always formed just beyond the endodermis and run for long distances throughout the root, (b) "secretory pockets," which are larger and shorter than the canals and the cavity of which is surrounded by secreting cells. He considered that these secretory pockets are reduced forms of canals, only differing essentially in longitudinal dimensions.

In 1900, Tschirch's important work on resin was published. Although he was, for the most part, concerned with the resin and resin ducts of Coniferae, his observations included some on the secretory canals of Composite roots. He concluded, as a result of this work, that resin formation took place in what he termed a "resinogener Schicht," *i.e.* a resin-forming layer, which he found lining the canal. He considered that the same layer was present in the case of the secretory canals of the Compositae, but in this case it was called a "Schleimschicht," or mucilage layer, and it was this layer that was supposed to excrete the resin into the canal. This conclusion has been challenged in a very recent paper by Moenikes who worked on the secretory canals of three families, Umbelliferae, Compositae, and Araliaceae. His work does not agree with Tschirch's conclusion, and he supports the theory that the secretion in the canals is due to the activities of the cells surrounding the cavity of the canal.

This brief review of the previous work on the subject will show that up to the present no special importance is attached to the position of the canals with regard to the endodermis (except in so far that they are stated to be formed after a division of the endodermal cells) and that this layer has been held to play no part in the secretion of the canal contents.

3. SECRETORY SYSTEM IN MATURE ROOTS

Up to the present time, the term "resin canal" has almost always been applied to these secretory canals found in Composite roots,

but it is a term which is open to some objections and is not altogether justified by the state of our knowledge on this subject. An obvious objection to the use of this term is that it seems to be quite uncertain whether the substance in the canals is a resin in the chemical sense of the word. The chemistry of resins is extremely complicated and has by no means been completely worked out. The term "secretory canal" seems preferable in many ways until more is known about the chemistry of the canal contents.

It is possible to classify these secretory canals into two main groups: A. Those formed immediately beyond the endodermis which will be termed "endodermal secretory canals." B. Canals whose formation is in no way connected with the endodermis; these will be called "non-endodermal secretory canals."

A. *Endodermal secretory canals*

The general appearance of the endodermal secretory canals in a mature root can be seen best by reference to Pl. III, fig. 1. They will be seen to be formed by intercellular spaces usually quadrangular in shape in transverse section, and they are filled with a semi-liquid substance, either colourless or yellow, which is invariably turned black by fixation in Flemming's strong solution. Longitudinal sections show that they run throughout the length of the root (Pl. III, fig. 3). The position of these canals is in reality extra-endodermal as they are enclosed, in their primary condition, by two endodermal cells and two cortical cells; they will, however, be termed "endodermal canals" for the sake of simplicity, and also to emphasise the fact that the endodermis is held to play an important part in their formation.

The mature canals may remain throughout their existence bordered by four cells which, in transverse sections, look exactly the same as the rest of the endodermal and cortical cells. In other cases, the canals become surrounded by thin-walled epithelial-like cells with dense cell contents, in every way suggesting meristematic cells. These may remain undivided, but in a large number of cases they undergo division; if this happens the canal increases largely in size and may come to be bordered by any number of cells up to about 10, e.g. *Buphthalmum speciosum*. The increase in size of the canal is accompanied by a corresponding increase in the amount of the contents which generally were found to fill the canal almost completely.

After the examination of a large number of plants, including examples from every sub-group of the family, it was found that

two extreme types of root could be distinguished and these types, which were connected to each other by a whole series of intermediate forms, could be associated with a difference in the growth habit of the root system.

The first type was characterised by the fact that the canals which formed each group remained distinct from each other throughout the life history of the root and the fatty contents of the root were distributed chiefly in the canals and on the walls of the external layers of the cortex. This was associated with a primary endodermis, and a wide regular cortex, and also with a small secondary development of the vascular system of the root so that comparatively little

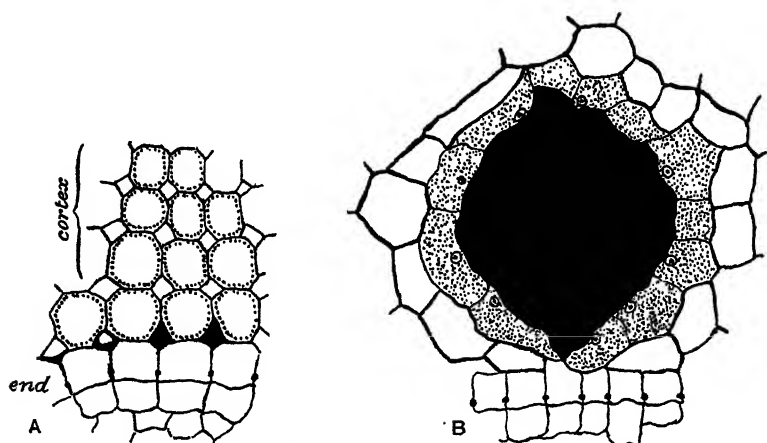


FIG. 1. A. A group of young endodermal canals of *Buphthalmum speciosum*.
B. A mature canal surrounded by epithelium-like cells.

strain can be exerted on the endodermis and cortex by the tissues within.

The following are a few of the plants which possess roots showing this structure: *Achillea ptarmica* (L.), *Buphthalmum salicifolium* (L.), *Cotula squalida* (Hook f.), *Doronicum plantagineum* (L.), *Gazania splendens* (Gaertn.), *Gerbera Jamesoni* (L.), *Liatris pycnostachya* (Michx.) and *L. spicata* (Willd.), *Senecio saracenicus* (L.), *Solidago virgaurea* (L.), *Stevia ovata* (Lag.), *Verbesina purpusii* (T. S. Brandegees) and *Vernonia novaeborascensis* (Willd.). Frequently, however, the development of secondary vascular tissue was such that the growth of the cortex was evidently unable to keep pace with that of the tissues within and the outermost layers of the cortex were ruptured and were gradually rubbed off. The cortex thus became narrower

in the older stages of the root, but the canals were unaffected and still remained distinct from each other. The walls of the outer cortical cells were suberised and, as these were removed, fresh fatty deposits were laid down below those on the extreme outside of the root. These roots appeared on the whole to be quite unable to form cork, although occasionally a few radial divisions had taken place in some of the cells just below the suberised outer layers. This inability to form cork may perhaps be due to the rapid increase in the vascular tissues and probably the constant rupturing of the outer cortical cells will not allow the conditions necessary for cork formation to be maintained for a long enough period for more than occasional cell divisions to take place in this region.

The second of the extreme types mentioned above was that of the majority of woody Composite roots. In the very young stages they resembled the herbaceous roots in structure, but the regular cortex never attained the same width as that of the herbaceous roots. Numerous divisions had taken place in these cells at an early stage and also in the endodermis itself, thus allowing the formation of secondary vascular tissues without at first putting much strain on the cortex. The endodermis generally became secondary and a complete ring of exogenous cork was formed in the most extreme types. The canals in this type of root structure showed a tendency to run together to form one or more larger ones. This, without doubt, was due to the strain ultimately developed on that region; the result of this was a stretching of the intercellular spaces, several of which were thrown together to form one large canal. The tangential stretching of the endodermis itself might lead to its rupture, especially in the regions of the canals, so that it no longer formed an impenetrable barrier. This was shown by the following experiment on *Artemisia lactiflora* in which the endodermis was in this stretched condition. Lead nitrate, which is not soluble in fat, was driven up lengths of root under a pressure head of about 20 cm. of mercury. The pieces of root were cut under water and the connections were also made under water. The presence of lead nitrate was detected by means of a glycerine solution of ammonium sulphide. The sections of the root were cut dry and were placed immediately in this solution. It was found, as a result of this experiment, that the lead nitrate had passed across the endodermis opposite the canals, thus showing a distinct leakage in this region. Roots possessing a complete secondary endodermis were found to retain the lead nitrate within the endodermal cylinder.

The activity of the cortical cells was a very marked feature of the roots of this group and several interesting cases were obtained showing that in older roots the principal cortical activity was localised round the canals, giving rise to the formation of groups of secondarily formed cortex consisting of radially arranged cells between the primary irregular cortex and the endodermis.

The intermediate stages in root structure could be traced along the following lines. Roots which formed very little secondary vascular tissue possessed a wide regular cortex, the outermost layer of which was suberised, and apparently it remained in this condition throughout the life of the root. When secondary vascular tissues were formed to a greater extent, the cortical cells towards the outside were ruptured and the suberisation of the outer cells moved progressively inwards as these were gradually cast off. In more woody roots, the cortex never attained the same width as that of the herbaceous roots, and divisions took place in every direction in these cells. Suberisation of the outer cell walls occurred and very often the outer layers of cells were not cast off or not nearly to the same extent as in the larger herbaceous roots. Finally, the formation of exogenous cork took place in the very woody roots either in patches or forming a complete cylinder round the root, e.g. *Artemisia lanata* var. *pedemontana* (Willd.), *Achillea argentea*, *Arctotis arborascens*, *Olearia Haastii* (Hook. f.), *Cosmos bipinnatus* (Cav.).

Non-endodermal Secretory Canals

These canals were usually to be found in the secondary phloem, but they may also be scattered throughout the medullary rays and cortex. They were formed, so far as could be seen from the available material, in older stages of the root only. For example, in *Rudbeckia Newmanii* they made their appearance in roots showing a considerable amount of secondary growth which had cast off some of the primary cortex and had formed exogenous cork in several places.

It has been difficult to get good preparations showing their formation owing to the rapidity of their development. Some examples were, however, obtained which showed the deposition of fatty substances between the walls of adjoining cells just before the appearance of the canal in this position. A certain amount of breaking down of the surrounding cells took place frequently when the accumulation of fat was excessive. They differed from the endodermal canals in that they were invariably short. The formation of epithelial-like cells surrounding the non-endodermal canals was

of common occurrence, although it was by no means always the case. They were often formed round the larger canals while they were absent from the smaller ones of the same root.

The factors controlling the formation of these canals have not been determined as yet, but they must be very different from those controlling the formation of the endodermal canals. From their

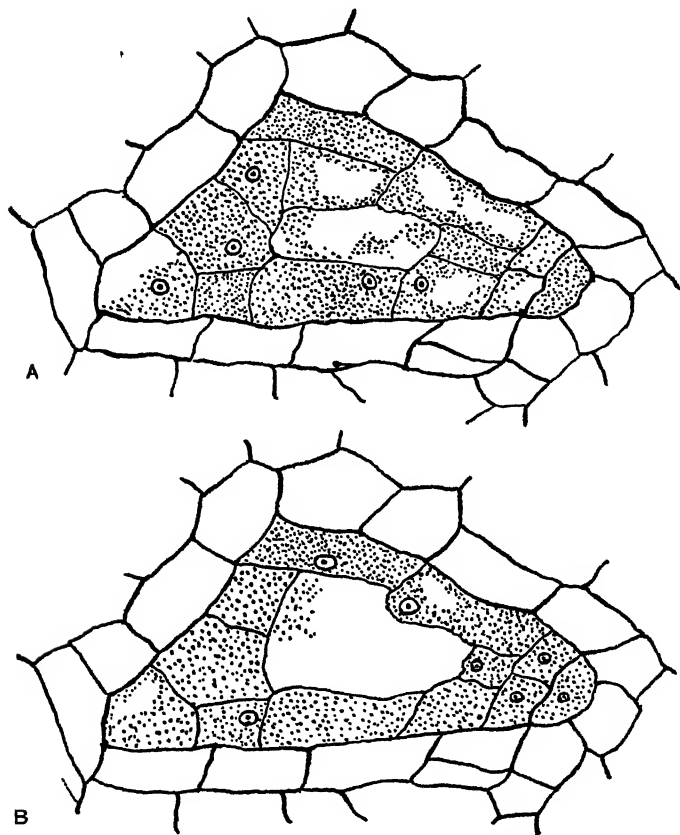


FIG. 2. A and B showing the schizo-lysigenous formation of non-endodermal canals in *Bidens dahlioides*.

sudden appearance and disappearance, it would seem that their formation might be due to some passing change in the metabolic equilibrium of the old stages of the root.

The following plants possess these schizogenously formed canals: *Centaurea babylonica*, *Rudbeckia Newmanii*, *Onopordon polycephalum*, *Senecio compacta*.

Other canals differing from those just described were found in *Bidens dahlioides* which forms tuberous roots similar to those of *Dahlia*. They often appeared to be schizo-lysigenously formed but resembled the above in extending longitudinally for a short distance only. In a series of microtomed sections of *Bidens dahlioides* the appearance of one of these canals was always preceded by an obvious group of epithelial cells with the usual dense cell contents and large nuclei. In some cases a small intercellular space could be detected in the centre of this group and then often in the course of a section or two, the canal was almost fully formed, consisting of a large cavity surrounded by secretory cells, and very often the remains of the broken down cells could be seen.

These canals were present throughout the parenchymatous ground tissue of the tuberous root, in the non-tuberous parts of the root they were formed in the cortex only. The non-endodermal canals of *Inula Helenium* originate in the same way, but they occurred only in the secondary phloem when a considerable amount of secondary growth had taken place.

4. THE DEVELOPMENT OF THE ENDODERMAL CANALS

The origin and development of the endodermal canals have received comparatively little attention in previous work on the subject, although comment has been made upon their early appearance. Triebel, in 1885, says, when writing about the formation of the endodermal canals in *Senecio sibirica*, that when the first xylem groups have been differentiated the endodermal cells divide tangentially; he goes on to say that the canals begin as minute intercellular spaces opposite the phloem groups at the junction of the radial and newly formed tangential wall. That is the most exact description of the origin of these canals in the literature on the subject and differs in several important details from the observations made on the origin of the canals in the following plants: *Solidago virgaurea* (L.), *Centaurea montana* (L.), *Stokesia cyanea* var. *alba* (L'Herit), *Helianthus annuus* (L.), *Inula glandulosa* (Lam.), *Inula Helenium* (L.), *Helenium autumnale* (L.), *Doronicum plantagineum* (L.), *Senecio saracenicus* (L.), *Bidens dahlioides* (S. Wats.) and *Verbesina Purpusii*. All the material was fixed in Flemming's strong solution which stains the contents of the canals black and therefore makes them very clear in the finished sections. Serial microtomed sections were made and they were stained usually with Gentian Violet and Bismarck Brown.

The first appearance of the canals in the root tip was in the region immediately behind the meristematic zone of the root. When

filled with fat, they stood out as minute black spots in one or two intercellular spaces opposite the phloem (Pl. III, fig. 2). They were present before there was any trace of differentiating xylem elements (which in Composite roots is formed some considerable time after the phloem) and also before the deposition of the Casparian strip on the radial walls of the endodermis. At this stage the cortex and the central conducting region were just marked out by a layer of cells—the future endodermis—beyond which quadrangular intercellular spaces occurred between the cells. All the cells contained dense protoplasts and large nuclei except the first differentiating sieve tubes which were the only vacuolating cells. They appeared as five-sided cells containing a small amount of watery looking protoplasm and were surrounded by cells which still retained their meristematic appearance. Immediately opposite these developing sieve-tubes and separated by two layers of cells was one intercellular space generally one layer further in than the rest of the intercellular spaces of the cortex. This usually contained fat, but in several cases they were found to be empty. According to Cramer in Bolles Lee⁽¹⁾ there can be no doubt as to the fatty nature of a substance which stains black with osmic acid after treatment with potassium bichromate; he says: "The presence of black cell globules in material fixed with bichromate osmic mixtures indicates the presence of true fats or cholesterin fatty-acid mixtures."

The discussion of the significance of the invariable occurrence of the canals opposite the first differentiating sieve tube will be left until a later stage, but it will probably make the subsequent sections clearer to state that the original contents of the canals are regarded as being of a fatty nature, most of which are derived from the developing phloem elements within the endodermal layer. It is difficult to see how the four cells surrounding the canal could have anything to do with the secretion of the fat at this early stage, as is suggested by Triebel and supported by Moenikes. They are obviously still in the meristematic stage like the rest of the cortical cells, and such a theory does not take into account the formation of the canals opposite the phloem—a phenomenon which is of universal occurrence throughout the family and presumably has some significance.

5. THE CHEMICAL NATURE OF THE CANAL CONTENTS

An attempt was made to get some evidence as to the chemical nature of the canal contents by means of microchemical and macrochemical methods.

The microchemical tests were found to be not altogether satisfactory on account of the relatively small amounts of the material present in the young stages and also because of the lack of distinguishing tests for fats, ethereal oils and resins.

Chemically, these substances are very different. A fat is an ester of glycerol with one or more of the higher fatty acids. Essential, or ethereal oils consist of a mixture of hydrocarbons, mostly terpenes, together with alcohols such as terpineol and camphors which are ketones of the formula $C_{10}H_{16}O$. The chemistry of resins is not nearly as well known as that of fats and ethereal oils. They are very complicated substances which are formed by a mixture of linkages of various kinds, and the majority of which contain phenol groups, aliphatic acids, aldehydes and terpenes, so that it can be readily seen that the detection of such a substance by means of single chemical tests would present a great deal of difficulty.

The following were the general tests used on all material, and as the results obtained over a very wide range of material were very uniform no special examples will be mentioned in connection with them. Later on, however, two special cases will be taken in detail to show how the results of the tests may differ in various stages of the root of the same plant. The canal contents in the youngest stages were found to be soluble in alcohol, ether and petrol ether. They went black immediately with a 2 per cent. solution of osmic acid and stained an orange red when warmed with Sudan III in a glycerine-alcohol solution and stained blue with Nile-blue sulphate. In the older stages, however, the canal contents were still very easily soluble in alcohol but ether and petrol ether had only a slight solvent effect. With osmic acid the black colour did not appear so quickly as in the young stages, and with Sudan III the colour obtained was a yellow red. Nile-blue sulphate was not taken up by the canal contents in these older stages. Unfortunately it is impossible to distinguish between fats, ethereal oils or resins by means of the above tests. Ethereal oils are however distinguishable from the other two substances by their solubility in acetic acid and chloral hydrate and also by their behaviour in the presence of the vapour of hydrochloric acid. If sections mounted in glycerine are left in an atmosphere of hydrochloric acid vapour, ethereal oils are driven off while fats remain in drops. As a result of these tests, it was found that the contents of the young canals remained insoluble in either of the solvents mentioned above and gave somewhat brownish drops after treatment with hydrochloric acid vapour instead of the typically

colourless or pale yellow liquid in the original canals. Far more solvent action with acetic acid and chloral hydrate was obtained in the case of the older canals and it was almost invariably found that a large amount of the contents were lost on treatment with hydrochloric acid vapour after which the canals were lined with a brown substance.

The only distinguishing test for resins mentioned in micro-chemical literature is that with copper acetate, which is very unsatisfactory in many cases owing to the length of time that may elapse before any action is obtained. Pieces of root are left in a concentrated solution of copper acetate for at least six days and the resin should become stained a bright emerald green. Tunmann⁽¹³⁾ mentions, however, that some resins only give the result after two months' treatment and others require an even longer time. No green colour was obtained in any of the roots tested in this way.

From the results of these general tests, the conclusion seemed to be justified that in all probability the basal substance in the young canals was a fatty substance soluble in alcohol, a conclusion which was supported by the following theoretical considerations. The canals are initiated just behind the growing apex and considerably in front of the absorptive region of the root; the food supply to the differentiating cells in this region must therefore be a downward stream. It is known that the cells of the apical meristem contain a large amount of fatty substances, particularly fats and phosphatides⁽⁷⁾, and also that the walls of the cells forming the phloem are peculiarly free from these substances. This suggests that the fats are completely lost from the differentiating phloem and that fat is typically absent from this tissue. The differentiation of a cell is accompanied by a loss of fat from the protoplast which would result in the appearance of the fat at the surface of the protoplast owing to its property of lowering the surface tension of any liquid with which it comes in contact. From there, it diffuses along the walls which at that stage will allow the passage of fat substances. Owing to sap pressures outward, it will pass to the endodermal layer at the junction of the cells of which are the first air spaces. Passing across the radial walls of this layer, it comes in contact with the intercellular spaces beyond, where it is deposited probably in an oxidised form. Evidently, at this early stage, the endodermal layer is incapable of picking up the fat to form the Casparian strip. This occurs later and is always formed first on the radial walls of those endodermal cells immediately opposite the phloem.

To conclude this section, a series of tests of *Buphthalmum speciosum* and *Heliopsis scabra* will be given to show the different results which are generally obtained in the young and the old stages of the canals.

In the young stages of *B. speciosum*, the secretory canals are of the normal form. In the older stages, however, the canals increase enormously in size and become surrounded by thin-walled cells (Fig. 1).

Results of Microchemical Tests on Buphthalmum speciosum

1. General tests.

The solubility of the canal contents was the same as those mentioned in the previous general tests, except that the contents of the young canals appeared to be slightly soluble in acetic acid. The other tests, *i.e.* Sudan III, 2 per cent. osmic acid and Nile-blue, gave the same results.

2. The action of heat on sections of the root.

The sections were heated very gently over a microburner. Part of the contents of the old roots disappeared, leaving a brown substance lining the canal. There was no action on the canal contents of the young roots.

3. With the vapour of concentrated hydrochloric acid as described on a previous page.

After this treatment the contents of the young canals became aggregated into brown drops. In the case of the old canals, part of the contents disappeared, leaving a yellow deposit and globules. Sections, after this treatment, were then washed several times in water to remove the hydrochloric acid. They were then heated gently in Sudan III, when the contents of the canals went a bright orange red.

4. Warmed in dilute hydrochloric acid solution.

From sections thus treated the same result was obtained as in the case of hydrochloric acid vapour. The hydrochloric acid extract was then evaporated down to dryness and small orange crystals were obtained which were found to be soluble in water, alcohol, and ether.

5. With iodine dissolved in potassium iodide (concentrated solution).

A dark brown solid was formed in the old canals, but no action took place in the case of the young canals.

6. With bromine water.

A dark brown non-crystalline compound was formed in the old canals.

7. With lead acetate.

The contents of the old canals went yellow.

8. With tannic acid solution.

A white to yellow solid was formed in the old canals.

The results of the above tests suggest again that the basal substance in the young stages of the canal is a fat. In the mature canals other substances are present some of which are volatile and probably include an alkaloid. The presence of the latter is suggested by tests 4 to 8.

The canals of roots at three different stages were examined in the case of *Heliopsis scabra*. The canals were of the usual type in appearance and in the youngest stage there was a wide cortex. In the next stage, a certain amount of secondary growth had taken place and the cortex was narrower. The canal contents were almost colourless in both these stages. In stage 3 the cortex contained thick walled cells towards the outside and the canal contents were a yellow brown.

Results of Microchemical Tests on Heliopsis scabra

1. General tests.

The contents of the canals of all stages were soluble in alcohol and insoluble in acetic acid, only those of the youngest stage were soluble in ether and petrol ether. With Sudan III, 2 per cent. osmic acid and Nile-blue, typical results were obtained, except that with Nile-blue all stages of the canals stained a pale blue.

2. The action of heat on sections.

Very little change could be detected in the canal contents at any stage.

3. With hydrochloric acid vapour for 24 hours.

The contents were unaffected in stages 3 and 2. In the youngest roots (*i.e.* stage 1) the contents formed yellow globules.

4. With iodine in potassium iodide.

The contents of the canals at all stages went dark brown.

5. With bromine water.

This reagent had no effect on the canal contents at any stage.

The results of these tests differ considerably from those obtained with *Buphthalmum speciosum*. In this case, very few volatile substances can be present and the contents of the canals give very similar results at all stages, suggesting that the original fat content

remains in a more or less pure condition throughout the existence of the root.

Macrochemical Tests

After a series of microchemical tests on a large number of genera, only two of which have been specially recorded in this paper, it was decided to try to obtain enough of the canal contents to perform some macrochemical tests. *Calendula officinalis* was considered to be the best plant to use. Preliminary microchemical tests with 2 per cent. osmic acid and Sudan III were done on fresh material giving results typical of young canals.

The rows of seedlings were thinned at intervals and the roots of the thinnings were cut off and were dried. The main crop was gathered when the seedlings were about three months old and these were treated in the same way. The roots were then boiled with absolute alcohol under a reflux condenser for about 12 hours. The alcohol was then poured off from the roots and was distilled off under reduced pressure until only a small quantity was left in the flask. The alcohol extract containing the canal contents, together with other substances, was then poured into a glass evaporating dish and the rest of the alcohol was allowed to evaporate off slowly under a bell-jar. The substance A left after the evaporation was complete was dark yellow brown in colour and more or less sticky. This was weighed and was next treated with petrol ether to remove the fatty substances. The petrol ether went a bright yellow colour almost immediately. The solid matter was allowed to settle and the petrol was pipetted off and allowed to evaporate away slowly in a weighed evaporating dish. The extraction with petrol ether was repeated several times until it was no longer coloured on being shaken up with the solid. When the petrol ether had evaporated, a yellow brown oily substance was obtained with an iodine number of 106.2. It was found that this drying oil formed about 20 per cent. of the substance A. The drying oil gave the following tests. It went black with 2 per cent. osmic acid solution and orange red when heated over a microburner in Sudan III. It rendered bromine water colourless, showing the presence of an unsaturated grouping, which is also proved by the possession of a high iodine number.

Substance B left after the extraction with ether was found to be practically completely soluble in water. The solution so obtained reduced Fehling's solution easily, and crystals of glucosazone were formed on treatment with phenylhydrazine hydrochloride in the

presence of acetic acid. Other substances besides glucose were undoubtedly present, but no further tests were performed as glucose evidently formed most of the water extract.

The fact that the drying oil formed 20 per cent. of the substances extractable with absolute alcohol provided good evidence that the contents of the secretory canals were being dealt with since the canals were the only part of the root which contained any bulk of alcohol soluble material. Additional evidence was afforded by the microchemical tests mentioned, the results of which were identical with those obtained from the treatment of the canal contents in fresh material.

It has been suggested by various writers that the canal contents may belong to one of three classes of chemical compound. It may be either an ethereal oil, a resin, or a fatty substance. A certain amount of evidence has been produced in this paper which shows that a volatile substance is present in some cases although absent in the same case in younger stages of the canal. Whether this volatile substance is an ethereal oil is still an open question, but the weight of the evidence is in favour of the opinion that, if present, it cannot represent the basal substance of the canal contents.

The chemistry of resins is still so uncertain that it is as yet impossible to identify them microchemically with any certainty. Moenikes(4), who supports the theory that the canal contents are resins, does so mainly on account of their solubilities and also because, in his opinion, he gets no reactions for fats and oils and also no soaps are formed on treatment with 20 per cent. solution of potassium hydrate. That no soaps were formed is not altogether surprising considering the small amount of material present in a section on which the potassium hydrate can act. It is difficult to see how the conclusion was arrived at that no fat or oil reaction was given by the canal contents, when one examines the list of solubilities and finds that the compound was soluble in alcohol-ether, ether, chloroform and also stained pale red with Sudan III. The solubilities of the canal contents represented those of certain fatty substances; also the staining reactions were those of fats as well as of resins. Finally when all the tests mentioned earlier in this section and the macrochemical experiments on *Calendula officinalis* are taken in conjunction with the theoretical considerations, it is held that the weight of the evidence is in favour of the presence of a fatty substance as the original basal substance of the canal contents.

6. MERISTEMATIC ACTIVITY IN THE CORTEX

It has been noted in a previous section of this paper that the cortex of Composite roots showed unusual meristematic activities in this region. In roots possessing a very wide regular cortex, the cells remained in an active condition for most of the life history of the root. The cells just beyond the endodermis divided by walls laid down in a radial direction and this took place first opposite the secretory canals. In roots showing rapid secondary developments of vascular tissue, the cortex was not active over such a long period, a fact which no doubt could be correlated with the use of the available food for these secondary developments. However, while this increase in the vascular tissues was beginning, the cells of the cortex underwent divisions in all directions, thus losing its very regular appearance. No examples were seen of fresh additions to the cortical cells just beyond the endodermis. In the more woody roots, it was often found that cortical activity was going on to some extent around the secretory canals, giving a semicircle of radially arranged cells in this region.

Another case of perhaps special interest in connection with the cortical activities was the formation of exogenous buds in the roots of some members of this family. Various species of *Gaillardia* have been found to give buds quite easily from their roots, especially *Gaillardia grandiflora*. Sections through these bud-producing roots showed that the buds were in nearly all cases formed exogenously and also that they were formed opposite the secretory canals. Only one case of endogenous bud formation was found, but in this case the bud was formed in a region of the root in which secretory canals happened to be absent. Whether this fact could be correlated with endogenous bud formation is a matter of conjecture, but there certainly are some grounds for such a conclusion.

Bud formation in the majority of roots of other families is endogenous, a fact which is undoubtedly bound up closely with the power of the secondary endodermis to prevent the passage of soluble food materials to the cortex except through passage cells when these are formed. Bud formation in itself necessitates the accumulation of much food in one place, and it follows from a consideration of the structure of such roots that this place must therefore be within the endodermis. The presence of an active and comparatively long-lived cortex in Composite roots together with the presence of a primary endodermis in *Gaillardia grandiflora* indicates that a much

more efficient food supply is supplying the cortex than in normal dicotyledonous roots. The fats and fat-soluble materials originating from the phloem could have no difficulty in passing across the Casparian strip which also consists of fat and it would form an additional food supply in the region of the canals. The accumulation of sufficient food in this region could then be followed by bud formation.

There is however one case, *Gazania splendens*, which showed that exogenous bud formation does not always take place in roots of this type. In this plant bud formation has so far taken place endo-

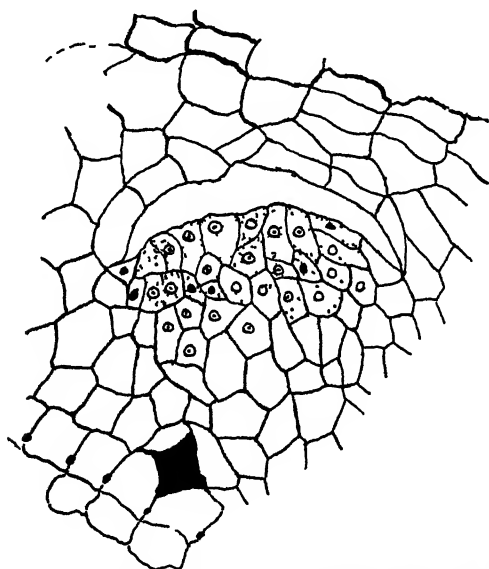


FIG 3 A young exogenous bud of *Gaillardia grandiflora* formed in the cortex opposite to a secretory canal

genously although no case has yet been found in which the bud has penetrated the endodermis. Exogenous bud formation also takes place in *Cirsium arvense* and *Helenium autumnale* var. Riverton Gem.

The frequent formation of exogenous cork has already been mentioned in a previous section and only a brief reference is necessary here. It is an instance of cortical activity occurring more especially in the woody roots when secondary thickening is well established. Cortical cell divisions in this case were confined almost entirely to the external regions of the cortex where a short series of radially arranged cells were cut off which very soon became suberised (Pl. III,

fig. 4). The cortex of the roots which formed exogenous cork in their later stages never attained, in the young stages, the width of cortex of the herbaceous roots, whose only protection on the outside was often a single suberised layer of cells.

7. GENERAL DISCUSSION

The most important point which comes up for discussion is the way in which the secretion reaches the endodermal canals. Two different ways have been suggested previously and a third is put forward in this paper. The three suggestions are as follows:

1. That it is secreted into the canal by the cells surrounding it.
2. That it is secreted into the canal by a mucilage sheath surrounding the canal cavity.
3. That the raw materials for the secretion originate in the very young and differentiating phloem; they then pass across the radial walls of the layer which will form the endodermis. These raw materials are not held back in the wall but pass on to the adjoining intercellular space where they are deposited in a combined and oxidised form. The presence of a Casparian strip will form no barrier to the passage of the secretion which, since it is a fatty substance of some kind, will be soluble in it.

The second of the theories mentioned is that brought forward by Tschirch principally in connection with work on resin canals in Coniferae. It has been attacked by several subsequent workers, amongst whom Hannig (1922) and Franck (1903) may be mentioned. No evidence could be found to support such a conclusion in the case of the coniferous resin canals. The theory when applied to the endodermal canals of the Compositae is equally untenable and has not yet received any support from any published work since it was originally put forward.

The first theory, *i.e.* that the secretion is formed in the surrounding cells and is secreted by them into the cavity, was suggested tentatively by Triebel⁽¹²⁾ in 1885. The suggestion has been accepted and has received support in a recent paper by Moenikes⁽⁴⁾. Triebel found that the canals of the species examined by him were surrounded by meristematic-looking cells which therefore might play an important part in the filling of the canals. Moenikes also found these meristematic-looking cells in the plants mentioned in his paper. He found that these cells contained globules which stained in the same way as the canal contents; these globules were distributed throughout the protoplast and were generally very small, needing

an oil immersion lens to be detected. Moenikes was here dealing with mature roots, an important point because these epithelial cells are formed in the older stages of the canal in these cases.

Although, after an examination of mature roots only, the natural conclusion to draw from such material might be that the canal contents were excreted from the epithelial cells, that conclusion does not receive any support from the facts presented by an examination of the initial stages of canal formation. In the first place why, if the surrounding cells are responsible for the contents, are these canals formed opposite the phloem just beyond the endodermis, and simultaneously with the differentiation of the first sieve-tube? That fact alone, surely, points to the conclusion that the canal contents are in some way a product of the developing phloem. Secondly, the canals in all the root tips so far examined, are first formed when the surrounding cells are similar, microscopically, to those of the rest of the cortex, and these surrounding cells contain no globules similar to those in the canals. Thirdly, the surrounding cells in mature roots are by no means always epithelial in nature and if present are nearly always formed in the old roots, and no signs of similar contents to those of the canals can be detected in a very large number of cases. It has been pointed out in a recent paper⁽⁸⁾ that the formation of meristematic cells from previously non-meristematic cells may be due to the presence of a gradient of hydrogen-ion concentration across them. Such a formation is constantly occurring in the cells surrounding the secretory canals which in their young stages are similar to ordinary cortical cells and later become meristematic in appearance and undergo division. Priestley and Woffenden⁽⁹⁾ showed that in the case of the wound healing of plants, the accumulation of fatty acids at the surface of the block produced a gradient to the normal parenchyma. On the same principle, the accumulation of fatty substances, together with other substances present at a later stage and often slightly acid in nature, might produce a similar hydrogen-ion gradient to the surrounding parenchyma in the later stages of the canal. If this were so, it would account for the fact that in the majority of cases these meristematic cells appear only at the mature stages of the canal.

The source of the canal contents of the non-endodermal canals is entirely different from that of the contents of the endodermal canals, and very little evidence is available as to the mechanism of their production. They are formed in the older roots only and appear to represent a local accumulation of fatty materials.

The presence of a primary endodermis throughout the life of the roots of many members of this family and of a peculiar oily secondary endodermis, when this is formed, is held to be very closely bound up with the cortical activities so often found in these roots. Such an active cortex must be supplied with an efficient food supply from the vascular cylinder and this is made possible by an endodermis of either of the two types found in these roots. In the first case, the passage of water soluble food materials is under protoplasmic control and fat soluble materials—apparently a very important part of the food supply, judging by the quantities of fat found in the roots at various times—can pass through the Casparian strip which is formed later than the canals and of the same materials. The formation of canals in this position will prevent the access of air to the fat held in the Casparian strip and will so prevent the formation of the rigid varnish-like strip of typical dicotyledons. The diffusion of fat-soluble materials is still possible when the secondary condition has been reached owing to the unusually liquid state of the suberin layer in this family; this prevents the drying up of the cortex which invariably takes place in the majority of roots.

8. SUMMARY

1. This paper is an attempt to gather up the published facts connected with the secretory system of Composite roots, to add some new ones, and also to interpret these facts in the light of recent work on the endodermis.

2. The various types of secretory canals found in the Compositae (Tubuliflorae section) are classified into two main groups:

A. Endodermal canals which are extra-endodermal in position, but their formation is closely bound up with that of the endodermis.

B. Non-endodermal canals, which show no connection with the endodermis and are developed in the older stages of the root.

3. The origin of the secretory canals at the root-tip in certain cases is described. It is shown that they originate at a very early stage and simultaneously with the differentiation of the first sieve-tube, being invariably formed opposite the phloem.

4. The chemical nature of the contents of the canal is discussed. The microchemical and macrochemical tests are given. It is concluded on the evidence of these tests and supported by theoretical considerations that the canal contents in the original stages consists of a fatty substance of the nature of an unsaturated drying oil. In

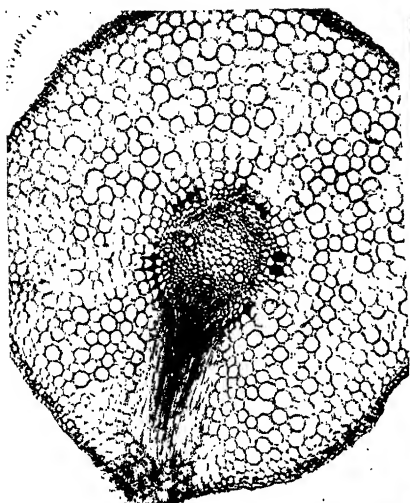


Fig. 1.

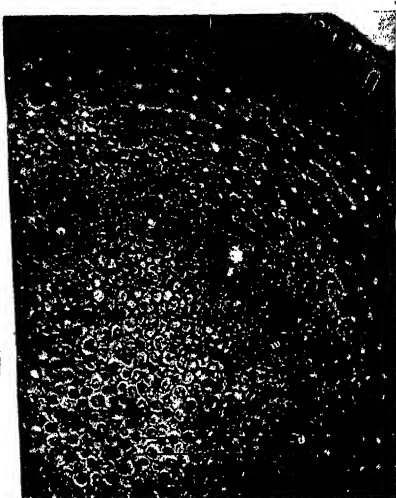


Fig. 2.



Fig. 3.

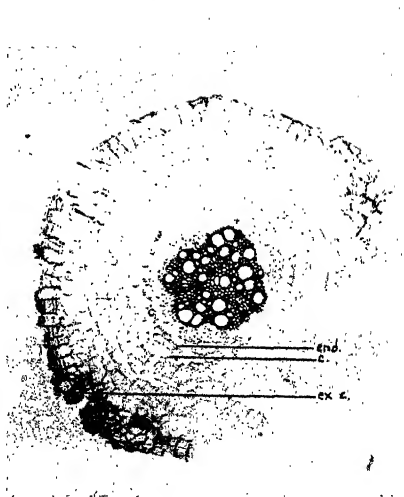


Fig. 4.

the older canals, other substances may be present, but probably all of these will be found to be fat soluble.

5. An account is given of the meristematic activities which take place in the cortex of these roots. It is shown that this is probably closely bound up with the presence of a primary endodermis throughout the life of most of the herbaceous roots, also with peculiarities to be found in the secondary endodermis when that is present.

6. Various theories connected with the method of deposit of the secretion in the canal are discussed. It is concluded that in all probability the fat is released during the differentiation of the phloem and from there it passes outwards and crosses the endodermis *via* the radial walls and is deposited in the endodermal canals.

In conclusion, I should like to take this opportunity of thanking Professor J. H. Priestley for his readiness in giving me the benefit of his experience and his advice throughout this investigation. My thanks are also due to Dr Bateson for working facilities provided at the John Innes Horticultural Institution, to the Director of Kew Gardens who provided me with many of the foreign members of the Compositae to which I should not otherwise have had access, and also to Dr Rhodes, of this Department, for his help in the chemical section of this paper.

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EXPLANATION OF PLATE III

- FIG. 1. Hand section of *Senecio saracenicus* showing a wide regular cortex and the endodermal secretory canals opposite the phloem.
- FIG. 2. Microtomed section of root of *Solidago virgaurea* just behind the root tip showing the formation of the secretory canals opposite the first developing sieve-tubes. *s.t.* = sieve-tube. *c.* = secretory canal.
- FIG. 3. Longitudinal section of the root of *Centaurea montana* showing the endodermal secretory canals.
- FIG. 4. Hand section of *Aryctotis arborescens* showing empty endodermal secretory canals and exogenous cork. *end* = endodermis. *c.* = secretory canal. *ex.c.* = exogenous cork.

THE ENZYMES OF *STEREUM PURPUREUM*

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A SURVEY of the enzymes of the fungus *Stereum purpureum* has been made at the suggestion of Mr F. T. Brooks, to whom the author is much indebted for helpful advice and criticism. The author is also indebted to the Hon. Mrs Onslow for advice on some of the tests.

The fructifications of this fungus were collected from various sources in Cambridge. Some of the material was used while still moist and leathery, but most of it was allowed to dry up in the laboratory, portions being subsequently moistened for a time before being used in the experiments. The fruit-bodies resume spore production after considerable periods of desiccation.

Pure cultures of the fungus were also made on a fairly large scale to provide mycelium for these enzyme tests. A thin agar plate culture was made from a spore deposit so that the fungal hyphae grew well away from bacterial colonies. From the tips of these

hyphae pure cultures were obtained. The medium was that used by W. Brown¹, and consists of

Glucose	2	grams
Asparagin	2	"
Starch	10	"
K ₃ PO ₄	1.25	"
MgSO ₄75	"
Water	1000	c.c.

300 or 400 c.c. of this medium in a 1000 c.c. flask with a few plum twigs stripped of their bark were autoclaved twice at 130° C. before inoculation. The mycelium of these pure cultures was used for enzyme tests after about three months' growth.

METHODS OF EXTRACTION

A. Fructifications

About 10 grams of dried fructification were dried and then:

(I) When testing for carbohydrate-splitting enzymes the tissue was minced in a juice extractor, and was then treated with alcohol to remove some of the cell-contents, especially reducing substances, and to precipitate the enzymes. The residue, after filtering and allowing the traces of alcohol to evaporate, was used direct or extracted with water in the incubator (29° C.) for a day or more. The enzymes then went into solution and could be separated from the tissues.

(II) When testing for oxidase and certain other enzymes the tissue was pounded in a mortar. The mush was used as such or a water extract was made as above. The water extract usually became dark; blood charcoal was once used to clear it, but this procedure caused the removal of the enzymes.

B. Pure-culture mycelium

The fungus material was transferred to a mortar and pounded up. The mush was

- (1) used direct,
- (2) extracted with water at 29° C. as above,
- (3) extracted with alcohol as above and the resulting dried mat of tissue was used direct or extracted with water.

Diastase.

I. *Pure-culture mycelium.* A water extract after treatment with alcohol was divided into three portions:

¹ Of the Botany Department, Imperial College of Science and Technology, London.

- (1) Fungus extract + 50 c.c. of 2 % starch "solution."
- (2) Fungus extract boiled + 50 c.c. of 2 % starch "solution."
- (3) Fungus extract alone.

Drops of (1) and (2) were taken out at intervals and tested with iodine solution, when it was found that after about 24 hours (1) ceased to give the blue reaction for starch.

When tested for reducing sugars, after a longer interval, with Fehling's solution 14 c.c. of (1) decolorised 20 c.c. of Fehling's solution whereas 13 c.c. of (2) did not, and further required 33 c.c. of standard invert sugar to decolorise the Fehling's solution.

By a separate titration 20 c.c. of Fehling's \equiv 34.5 c.c. of invert sugar.

So (1) \equiv 34.5 c.c. of invert sugar,

(2) \equiv 1.5 " " "

Diastase is therefore present in the pure-culture mycelium.

I. *Fructification.* The residue after treatment with alcohol was used dry; 2-3 c.c. of distilled water were added to each portion of the residue and one portion was boiled.

- (1) 1 gram of fungus-meal + 10 c.c. of 2 % starch "solution."
- (2) 1 gram of fungus-meal boiled + 10 c.c. of 2 % starch "solution."

When tested with Fehling's solution in the same way as the mycelium, the results were as follows:

(1) \equiv 49 c.c. of standard invert sugar.

(2) \equiv 3 " " " "

Diastase is therefore present in the fructification.

Glycogenase.

Very little glycogen was available and an approximately 0.5 per cent. solution was used.

Fructification. A water extract after alcohol.

- (1) 10 c.c. of fungus-extract + 10 c.c. of glycogen solution.
- (2) 10 c.c. of fungus-extract heated at 100° for 10 minutes + 10 c.c. of glycogen solution.

These were incubated at 29° C. for 2-3 days.

At the end of this time a drop from each was tested with iodine solution when

- (1) gave no colour,
- (2) gave a brownish red colour (due to glycogen).

When tested with Fehling's solution the results were as follows:

(1) \equiv 19.8 c.c. of standard invert sugar,

(2) \equiv 13.4 " " " "

The glycogen solution alone had no effect on Fehling's solution.

By a separate titration 10 c.c. of the fungus extract alone was found equivalent to 11.6 c.c. of standard invert sugar. Subtracting this figure from each result gives

(1) \equiv 8.2 c.c. of standard invert sugar,

(2) \equiv 1.8 " " " "

which shows that glycogenase is present in the fructification. This enzyme is apparently not entirely deactivated by heating at 100° for 10 minutes.

Inulase.

Fructification. A water extract after alcohol.

(1) 10 c.c. of fungus extract + 10 c.c. of 2 % inulin.

(2) 10 c.c. of fungus extract heated at 100° for 10 minutes + 10 c.c. of 2 % inulin.

When tested with Fehling's solution after 2-3 days' incubation at 29° C. (1) and (2) required 10.5 c.c. and 10.7 c.c. of standard invert sugar respectively to complete the titration.

Inulase therefore is not present in the fructification.

Invertase.

I. *Fructification.* A water extract after alcohol.

(1) 10 c.c. of the fungus-extract + 20 c.c. of 2 % cane sugar.

(2) 10 c.c. of the fungus-extract boiled + 20 c.c. of 2 % cane sugar.

After two days at 29° C. each was boiled with 20 c.c. of Fehling's solution.

(1) reduced the whole of it and part of another 20 c.c. of Fehling's solution,

(2) reduced a small amount.

Invertase therefore is present in the fructification.

II. *Pure-culture mycelium.* The residue after treatment with alcohol was divided into two equal parts. 2-3 c.c. of distilled water were added to each, and one was boiled.

(1) unboiled + 20 c.c. of 2 % cane sugar,

(2) boiled + " " " "

When tested with Fehling's solution after 2-3 days at 29° C.: 10 c.c. of each were added to 20 c.c. of boiling Fehling's solution:

(1) required 11 c.c. of standard invert sugar to complete the reduction,

(2) required 30 c.c. of standard invert sugar to complete the reduction.

20 c.c. of Fehling's solution was \equiv 34 c.c. of standard invert sugar. Invertase therefore is present in the pure-culture mycelium.

Maltase.

Maltose itself is a reducing sugar but the Fehling's test can be used for maltase since the amount of monosaccharide obtainable on hydrolysis has theoretically double the reducing power.

I. *Fructification.* The solid residue after alcohol.

- (1) 2 grams of fungus meal + 20 c.c. of distilled water + 20 c.c. of 2 % maltose,
- (2) 2 grams of fungus meal + 20 c.c. of distilled water boiled + 20 c.c. of 2 % maltose.

When tested with Fehling's solution after 2-3 days at 29° C.:

10 c.c. of (1) required 13 c.c. of standard invert sugar to complete reduction,

10 c.c. of (2) required 21 c.c. of standard invert sugar to complete reduction.

So the reducing power of (1) and (2) are in the ratio of 1.6 : 1.0. Maltase therefore is present in the fructification.

II. *Pure-culture mycelium.* A similar experiment to that with the fructification gave a ratio of 1.4 : 1.0. Probably the mycelium contains the enzyme since the results are in the right direction and the fructification contains it. Further experiments would no doubt give a more direct answer.

Raffinase.

Fructification. A water extract after alcohol.

- (1) 10 cc. of the fungus extract + 10 c.c. of 2 % raffinose.
- (2) 10 c.c. of the fungus extract boiled + 10 c.c. of 2 % raffinose.
- (3) 10 c.c. of the fungus extract alone.
- (4) 10 c.c. of 2 % raffinose alone.

After five days at 29° C. these were tested with Fehling's solution and gave the following values in terms of standard invert sugar

- | | | | |
|-----------|-----------|-----------|--------|
| (1) 51.2. | (2) 21.2. | (3) 20.6. | (4) 0. |
|-----------|-----------|-----------|--------|

The value for the fungus extract alone shows that hydrolysis of the raffinose has not occurred in the boiled control. Raffinase therefore is present in the fructification.

Pectinase.

As substrate, discs of potato tuber 20 mm. in diameter and .32 mm. in thickness cut with a hand microtome were used. A number were cut fresh for each experiment and selected for uniformity.

I. *Fructification*. Water extract after alcohol.

(1) extract + 3 potato discs .32 mm. thick,

(2) boiled extract + 3 potato discs .32 mm. thick.

After 24 hours a disc from each was tested for coherence by placing it on a slide with water and pulling with the fingers. The discs from (1) came apart more easily than those from (2). After 48 hours the discs from (1) were difficult to handle without tearing, while those from (2) remained coherent.

Pectinase therefore is present in the fructification.

II. *Pure-culture mycelium* water extract after alcohol filtered and unfiltered.

(1) mush + 3 discs of potato tuber .32 mm. thick.

(2) boiled mush + 3 discs of potato tuber .32 mm. thick,

(3) filtered extract + 3 discs of potato tuber .32 mm. thick.

(4) boiled " " " "

After 24 hours (1) and (3) test discs came apart easily.

After 24 hours (2) and (4) test discs remained coherent.

After 48 hours (1) and (3) test discs less coherent than after 24 hours.

After 48 hours (2) test disc was somewhat less coherent than at the beginning but (4) test disc unchanged.

Pectinase therefore is present in the mycelium.

Emulsin.

I. *Fructification* pounded in a mortar with thymol and toluol and left covered in a Petri dish for nine days. Then it was extracted with water at 29° C. for an hour and filtered. The filtrate was light brown. "Picrate paper" was used to demonstrate the liberation of HCN. The method of preparation is given in *Practical Plant Biochemistry*(5), p. 146:

(1) 10 c.c. of fungus extract + 20 c.c. of 2 % amygdalin solution.

(2) 10 c.c. of fungus extract boiled + 20 c.c. of 2 % amygdalin solution.

(3) 10 c.c. of amygdalin solution alone.

A strip of picrate paper was suspended in each flask above the liquid, being held by the cork.

In 12 hours the picrate paper in (1) had turned red while those in (2) and (3) were unchanged.

II. *Pure-culture mycelium* water extract after alcohol.

(1) 20 c.c. of extract + 20 c.c. of 1 % amygdalin + picrate paper.

(2) 20 c.c. of extract boiled + 20 c.c. of 1 % amygdalin + picrate paper.

(3) Amygdalin alone + picrate paper.

Examined after three days at 29° C.

(1) orange, (2) no change, (3) slight colour.

Emulsin is therefore present in the fructification and the mycelium.

Protease.

I. Fructification

(a) water extract after alcohol:

(1) 10 c.c. of extract + 10 c.c. of flour extract.

After three days at 29° C. no reaction for tryptophane.

(b) solid after alcohol:

(1) 2 grams of fungus meal + 30 c.c. of 2 % peptone.

(2) 2 grams of fungus meal heated + 30 c.c. of 2 % peptone.

After five days at 29° C. no reaction for tryptophane.

(c) fructification ground to a watery mush and used as a liquid.

(1) 15 c.c. of this liquid + 24 c.c. of 1 % peptone.

(2) 15 c.c. of this liquid boiled + 24 c.c. of 1 % peptone.

No reaction for tryptophane.

Gelatine slants however were liquefied at room temperature by the fungus mush in the presence of thymol and toluol.

II. Pure-culture mycelium.

(a) water extract:

(1) 10 c.c. of extract + 10 c.c. of flour extract.

(2) 10 c.c. of extract boiled + 10 c.c. of flour extract.

After three days at 29° C. no reaction for tryptophane.

(b) solid after alcohol:

(1) a portion of the mycelium + 10 c.c. of 2 % peptone.

(2) a portion of the mycelium heated + 10 c.c. of 2 % peptone.

After five days at 29° C. no reaction for tryptophane.

To test for tryptophane, bromine water was added drop by drop when a pink colour should appear if tryptophane is present.

As a control this test was performed on a solution of tryptophane, and a positive result was obtained with it when diluted 10 times.

The fungus therefore lacks an enzyme capable of hydrolysing peptone to give tryptophane. On the other hand, it has an enzyme capable of liquefying gelatine.

Oxidase.

Fungus material treated with alcohol did not give any positive results.

I. Fructification. Some fruit bodies were taken from a laburnum log and pounded with a pestle and mortar while still moist; water

was added and mixed with the mush, and small quantities of the liquid were poured into three porcelain cups.

- (1) three times as much water added + 1 c.c. guaiacum tincture.
- (2) three times as much water added + 1 c.c. guaiacum tincture + 3 drops H_2O_2 (10 vol.).
- (3) boiled and then treated as in (2).

The liquid in (1) turned blue in 15 minutes.

The liquid in (2) turned blue in 2-3 minutes.

The liquid in (3) was not coloured but the edges of solid particles of the fungus tissue turned blue in about 20 minutes.

Another experiment was performed using 1 % benzidine solution in 50 % alcohol as indicator.

- (1) Same treatment as (1) above but 2 c.c. of benzidine solution substituted.
- (2) Same treatment as (2) above but 1 c.c. of benzidine solution substituted.
- (3) Same treatment as (3) above but 1 c.c. of benzidine solution substituted.

The liquid in (1) gave no colour in 15 minutes.

The liquid in (2) turned blue in 2-3 minutes.

The liquid in (3) gave no colour, but colour appeared on the solid pieces of tissue as in (3) above.

A complete oxidase system is therefore present in the fructification.

II. *Pure-culture mycelium.* Some mycelium was taken fresh from a culture flask and pounded in a mortar. Water was added and the larger particles of tissue allowed to settle. The liquid was poured off and used in the same way as in the experiments with the fruit bodies.

The unboiled extract blued guaiacum tincture in the same period of time as the extract from the fruit bodies, with a similar acceleration with H_2O_2 . The boiled extracts, however, gave no colour.

Extracts have been made from fruit bodies for several days with water and toluol, which were more powerful, turning guaiacum tincture blue in five minutes without H_2O_2 . These extracts throw down a precipitate when heated above $80^\circ C.$, which temperature does not destroy the bluing power. When the heated extract was tested, some of the precipitate was always included in the sample. It is only in this precipitate that the blue colour appears. Even when heated at $100^\circ C.$ for 10 minutes (by dipping the test-tube in an oil bath at $110^\circ C.$) the blue colour appeared within an hour.

Mycelium extracts made in the same way throw down a precipitate when heated but do not then blue guaiacum tincture.

Tyrosinase.

I. *Pure-culture mycelium.* Water extract after alcohol, some filtered and some used direct.

- (1) Unfiltered + tyrosin suspension in a corked tube 2 inches long and $\frac{1}{8}$ inch wide.
- (2) Unfiltered boiled + tyrosin suspension in a corked tube 2 inches long and $\frac{1}{8}$ inch wide.
- (3) Filtered extract + tyrosin suspension in a corked tube 2 inches long and $\frac{1}{8}$ inch wide.
- (4) Boiled extracted + tyrosin suspension in a corked tube 2 inches long and $\frac{1}{8}$ inch wide.

The mush at the top of tube (1) became dark in 24 hours.

The liquid in tube (3) went dark all through in 24 hours.

The contents of tubes (2) and (4) remained colourless.

Tyrosinase therefore is present in the pure-culture mycelium.

Catalase.

Whenever hydrogen peroxide was added to fresh fungus preparations (fructification and cultural mycelium) a vigorous effervescence took place. No gas was evolved from boiled preparations. The gas was collected in a small tube and shown to be oxygen. Catalase therefore is present in the fructification and the pure-culture mycelium.

Reductase.

Methylene blue as substrate.

Fructification and pure-culture mycelium

Only water extracts after alcohol were tried and the negative results may be due to the enzyme being destroyed by the alcohol in the same way as the oxidase.

In similar experiments zymine decolorised the methylene blue preparation in 30 minutes.

DISCUSSION

Recent work by Buller (1), Dodge (2), Dox (3), Harter and Weimer (4), Zeller (5) indicates that there is a great variety of enzymes present in fungi, and this investigation shows that *Stereum purpureum* contains a number of these bodies. It is noteworthy that some of

the enzyme preparations were not easily inactivated by heat, and that the only difference as regards enzymes between the fructification and the pure-culture mycelium lies in the greater susceptibility of the mycelium to heat. The presence of solid particles of fungus tissue seems to have protected the oxidase and pectinase, and the formation of a precipitate in other experiments on oxidase may have acted in the same way. No account has been taken of the possible influence of other substances in solution on the protection or the inactivation of enzymes when fungus extracts were boiled, so these suggestions are tentative.

SUMMARY

A survey of the enzymes in the fungus *Stereum purpureum* has been made. Both fructifications and pure-culture mycelium have been used, and the active extracts have been prepared from moist living tissues.

The following enzymes have been tested for: diastase, inulase, glycogenase, invertase, raffinase, maltase, emulsin, pectinase, protease, tyrosinase, oxidase, reductase.

Positive results have been obtained for: diastase, inulase, glycogenase, invertase, raffinase, emulsin, pectinase, tyrosinase, oxidase.

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VIRESCENCE IN *DELPHINIUM*

BY R. R. GATES AND W. R. IVIMEY COOK

(With Plate IV)

VIRESCENCE is an abnormality which has often been reported in *Delphinium* and other forms, but notwithstanding the numerous records of its occurrence it has seldom been studied from the genetical point of view. Many other flower monstrosities, such as peloria, are of sudden origin and should no doubt be classed as mutations, but few of them have been subjected to breeding experiments. The present record is published in order to direct attention to the need for experimental work of this kind with teratological forms. The monstrosities should be crossed, if possible reciprocally, with the type, and at least two, or in many cases three generations of the hybrids should be grown to determine its inheritance. If the crossing is impossible owing to sterility or other causes, normal plants of the strain in which it appeared should be self-pollinated and their progeny examined. Its reappearance under these circumstances would furnish evidence concerning its manner of inheritance, and such simple experiments require no genetical experience.

Widening experience is showing that many semi-monstrous conditions in plants and animals, including man, are Mendelian in their inheritance. In the majority of cases they are not distortions or derangements environmentally produced and temporary in character, but they represent germinal changes and will be passed on to descendants. How frequently such cases are inherited can only be determined by experiments, but they probably represent the large majority of teratological malformations.

In June, 1924, a spike of *Delphinium* showing numerous flowers, all virescent, was brought into the Botanical Department by a former student. It appeared in a London garden among a number of normal plants. It was immediately photographed and then preserved in a jar as a museum specimen. An effort to obtain seed from the strain was unsuccessful, but a careful description of the form is desirable. In the light of other records of virescence, it is possible that some normal members of the strain would again have produced the virescent condition. The cultivated *Delphiniums* have been much hybridised, and apparently *D. consolida*, *D. Ajacis* and

D. elatum have entered most largely into these crosses. The specimen was identified at the British Museum as being nearest *D. elatum*, although not agreeing entirely with that species.

Pl. IV, fig. 1 shows the general habit of the branch. This illustration also shows the shape of the leaves, which was one of the chief characters used in the identification of the species, as the flowers were too much modified to give a clear indication, and the normal flowers were not available.

In *D. elatum* two bracteoles occur, which are situated on the peduncle a short distance below the sepals. These are opposite and of the same size; they are rather hairy and pointed at the tips. In the virescent form there are also two bracteoles, but they are not opposite nor of equal size; the longer is below the shorter and on the opposite side of the peduncle. In shape they resemble the normal form.

The flower of *Delphinium* is well known. Of the five sepals the posterior one is produced backwards to form a spur equal in length to the sepal. In the virescent form (Pl. IV, fig. 2) the sepals are very much larger, and are hooded and baggy in appearance. The spur of the posterior sepal develops very late, and only in quite mature flowers does it reach its full length, which is only about one-third that of the sepal. The spur is constantly forked at the end, a feature which is not known in the normal form. In the normal flower of *D. elatum* only the two posterior petals are present, and they have spurs which pass into the tube formed by the posterior sepal. As Knuth (1908) pointed out, the length of the spur precludes any except long tongued insects reaching the nectar at the base. In the virescent form the petals are very much reduced in size, but all five are present, and they are usually opposite the sepals instead of alternating with them. Moreover they are all alike and unspurred; they are also small and spatulate, about one-third the length of the sepals, and there is no indication of a nectary.

The androecium of the virescent flowers was perfectly normal in appearance, the filaments and anthers were of the ordinary size, and the pollen grains appeared unshrunk and viable. The gynoeceum of *Delphinium* normally possesses three carpels, except in the *Consolida* section, which has but one. The apices of the carpels are prolonged as short, straight styles. In both the virescent form and the type the styles were hairy on their inner surface. In the virescent form the three styles are prolonged into hooked ends which curved inwards to the centre of the flower (Pl. IV, fig. 3). The stigmatic

surfaces were also apparently not functional, so that although no modification of the ovules was observed, fertilisation would not take place.

DISCUSSION

Virescence, peloria and "doubling" in various forms are all conditions of widespread occurrence in flowers, and breeding experiments indicate that the large majority of such cases are mutations. The two former bear some relation to each other in the present case of *Delphinium*.

Peloria was first described by Linnaeus (1742) in *Linaria vulgaris*, being found in a wild plant. It is essentially a change from a zygomorphic to an actinomorphic flower, and appears in two forms: (a) nectaria, with every sepal or petal spurred, (b) anectaria, in which there are no spurs. Godron (1865) records a *Delphinium* with five petals and five spurred sepals. He compares this condition with that normally present in *Aquilegia*. In the present virescent *Delphinium* the corolla is of the peloric anectaria form, but the calyx remains of the zygomorphic type. Masters (1868) records virescence in *Delphinium*. Penzig (1921) also gives a number of cases in various species, but the details are not sufficient to determine whether any of these agree with the present case. Worsdell (1916) cites and carefully describes many cases of floral abnormality in *Delphinium*. The condition of peloria is now known in many zygomorphic families such as Scrophulariaceae, Labiatae, Leguminosae and Orchidaceae. The genetical studies of some of these are referred to elsewhere (Gates, 1921). It may be suggested here that the genus *Aquilegia* probably arose as a peloric mutation from an ancestor with zygomorphic flowers, and persists perhaps because the five nectaries gave an advantage in insect visits. The sudden change from one to the other condition is known to have occurred in fact in many genera. Worsdell (1916) indeed, cites a record of a *Delphinium Ajacis* flower with three sepal spurs, but the modern records of mutations show that the extreme form often arises directly from the type, the intermediates being merely independent derivatives from the same type.

The term virescence has been loosely applied to a variety of conditions, representing a great range of departure from the normal. The change involved in a single whorl of floral organs such as the carpels, may be quite different in kind in different cases. The simplest change in virescence consists only in a greening of the petals. A remarkable case of this kind has been described in *Oxalis*

stricta (Hus, 1907). Here the occurrence of chloroplasts in addition to chromoplasts was accompanied by minor changes in the petals, such as in the veining, the shape of the epidermal cells, and increase in thickness of the petals. This form was found wild near St Louis, Missouri, about a dozen virescent plants being found in an area of 8 square feet interspersed with normals. Seed collected from green-petalled plants yielded 43 with green petals, and one normal. The strain was taken to the experimental garden at Ann Arbor, Michigan (Hus, 1911), where it spread rapidly from self-sown seed, remaining constant for nine generations, although when grown in shade it was less green than in full sunlight. The variety also maintained itself in the Missouri Botanical Gardens. It is nevertheless surprising that it was found (Bartlett, 1909) in plenty growing wild on the edge of a pine barren at Thomson, Georgia, where it had evidently originated as an independent mutation. Incidentally such cases show the necessity for caution in drawing conclusions from the facts of distribution alone.

When the flower parts are not only green but are more or less leaf-like in form the term frondescence is sometimes applied. But many kinds of mutations are included here. The petals, sepals, stamens or carpels may be involved. The sepals are frequently larger, the petals smaller and in the more extreme types of virescence the stamens and carpels may also both be transformed into leaf-like and more or less completely sterile structures. Proliferation (*Durchwachsung*) frequently accompanies these conditions when the carpels are affected. There is need for a modern system of classification for these teratological flower types, the majority of which, it may be safely assumed, are inherited. Even when the flower is itself completely sterile in anthers and ovaries there is evidence that the same condition often reappears from normal members of the strain, and not in related ones, showing that it is represented in the germ-plasm.

It may also be pointed out that many virescent conditions of the flower are, from an ontogenetical point of view, failures to complete development. The same is true of many other malformations. A careful study of the development of virescent flowers from this point of view is desirable. It is possible that even the greening of petals is really due to a failure to transform the chloroplasts into chromoplasts.

Reference may be made to a virescent race of *Oenothera* which showed some striking similarities to the present case in *Delphinium*. This race was derived from seeds obtained from Birkenhead in 1908

(Gates, 1910). One plant from these seeds gave, when self-pollinated, 376 plants, 4 per cent. of which developed virescence. The earliest flowers were normal, but all the later flowers in virescent plants had characteristic baggy sepals and very much reduced petals, as in *Delphinium*. The anthers, however were also very small, on very short filaments and devoid of pollen. As in *Delphinium*, the style was pubescent and non-stigmatic, but it was also tapering above and with much reduced stigma lobes. Such flowers were therefore totally sterile. Other changes were in the suppression (more or less complete) of the hypanthium, and the absence of an absciss layer. Often the ovary and the elongated stalk below it developed into a woody branch, sometimes even with internodes, and in these cases usually developed leaves inside the petals. The condition was inherited in the next generation. Masters (1862) states that buds have been formed in the axil of the sepals in *Clematis*, *Delphinium*, *Caltha*, *Aconitum* and *Anemone*.

Under the name virescence, Stomps (1918) describes a very different condition occurring in a race of *Oenothera biennis*. There were no true flowers, but in the axil of every leaf was produced, in place of a flower, a group of small branchlets bearing small, narrow leaves. A flower of *Solanum Lycopersicum* is also described (Stomps, 1916) in which a whorl of six leaf-like parts represents the calyx, and the other parts of the flower are represented by small green rudiments.

A case of much genetical interest has been investigated by Simon (1924). In 1912 a virescent plant appeared in a normal white variety of *Torenia Fournieri*. Its flowers varied much. The calyx was almost entirely normal, but instead of a zygomorphic two-lipped corolla the individual petals developed more or less independently and irregularly. The filaments were often petaloid, and the anthers contained no good pollen. The style was short, and there was no stigmatic surface. In many flowers the ovules were exposed, through the carpels failing to unite. In the leaf axils a group of virescent flowers developed surrounded by tiny buds. This plant evidently combined certain abnormal features found separately in other cases already described. Descendants of this plant also showed *Durchwachsung* from abnormal flowers to form normal ones. An allied condition of proliferation in *Cardamine pratensis* has long been known, and has recently been described in detail by W. Robinson (1925). Here, in the absence of a replum, the base of the ovary takes on meristematic growth and ultimately gives rise to double flowers. After a succession

of such flowers, the growing point in the centre of some flowers begins to produce leaves, forming a tuft-like bud with roots near their base.

The virescent plant of *Torenia* was propagated by cuttings. These produced flowers some of which had a nearly normal stigma. They were pollinated from the normal form with violet flowers. The results cannot all be considered here, but the crosses gave 54 normal plants, 24 others (including 7 typically virescent). There were also some dwarfs, and some of a flower-type known as *brevischistostyle*. In later generations about a dozen distinct types appeared. The *brevischistostyle* plants are of interest because they clearly resemble, though they are not identical with, the *Oenothera* mutation which de Vries called *brevistylis*. The style is short and the stigma badly formed, as in *brevistylis*, but in addition the style is forked some distance down. *Oenothera brevistylis* is known to be a Mendelian recessive in inheritance. The *brevischistostyle Torenia* when crossed with the normal gave only normal F_1 (over 1000 plants) but in the F_2 a large number of forms appeared. This is interpreted to mean that a whole gene complex has mutated in the origin of this form. Simon points out that *T. Fournieri* was first described and brought into cultivation in 1876, and he believes it has never undergone crossing. The original colour was dark violet, but light violet and white mutations have since appeared.

It remains to point out that virescence, like many other variations, may also occur in a non-inherited form due to environmental impress. The best known instance was recorded by de Vries (1896). An epidemic of virescence occurred in his experimental garden at Amsterdam. Out of 80 species, 24 were affected. These included *Agrostemma*, *Githago*, *Silene noctiflora* and several Composites. The epidemic spread from one species to another, and the cause was traced to insect attack. Whether the bite of insects alone was the cause, or whether possibly it was accompanied by the transference of bacteria or other microorganisms, was not determined. In many cases such changes resemble galls. Goebel (1900) cites a paper of Peyritsch in which it is stated that he was able to induce phyllody in *Arabis* by infecting them with aphids, provided that the flower bud was not in too forward a state of development.

That the same teratological abnormality may be induced externally or may appear as the result of a germinal change is a fact of fundamental significance.

SUMMARY

1. A virescent inflorescence of *Delphinium* is described.
2. The flowers all have five large hooded sepals enclosing five very reduced petals, none of which are spurred. The posterior sepal has a short spur, which develops late, and is forked at the tip. The three carpels are prolonged into curved, non-stigmatic, hairy tips.
3. This case is compared with other records of virescence and peloria, and particularly with various instances in which genetic experiments have been made.
4. It is pointed out that many different kinds of change are included under the term virescence, and that the same teratological condition may appear as the result of a germinal change or as an externally impressed modification.

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Fig. 1



Fig. 2



Fig. 3

DESCRIPTION OF PLATE IV

- FIG. 1 The complete flower spike showing the character of the leaves, and the large number of mature flowers.
- FIG. 2. A single flower showing the five hooded sepals with the petals lying inside them. The anthers appear perfectly normal.
- FIG. 3 A single flower showing the *loking* of the spur of the posterior sepal, the curved styles and the hooded character of the sepals.

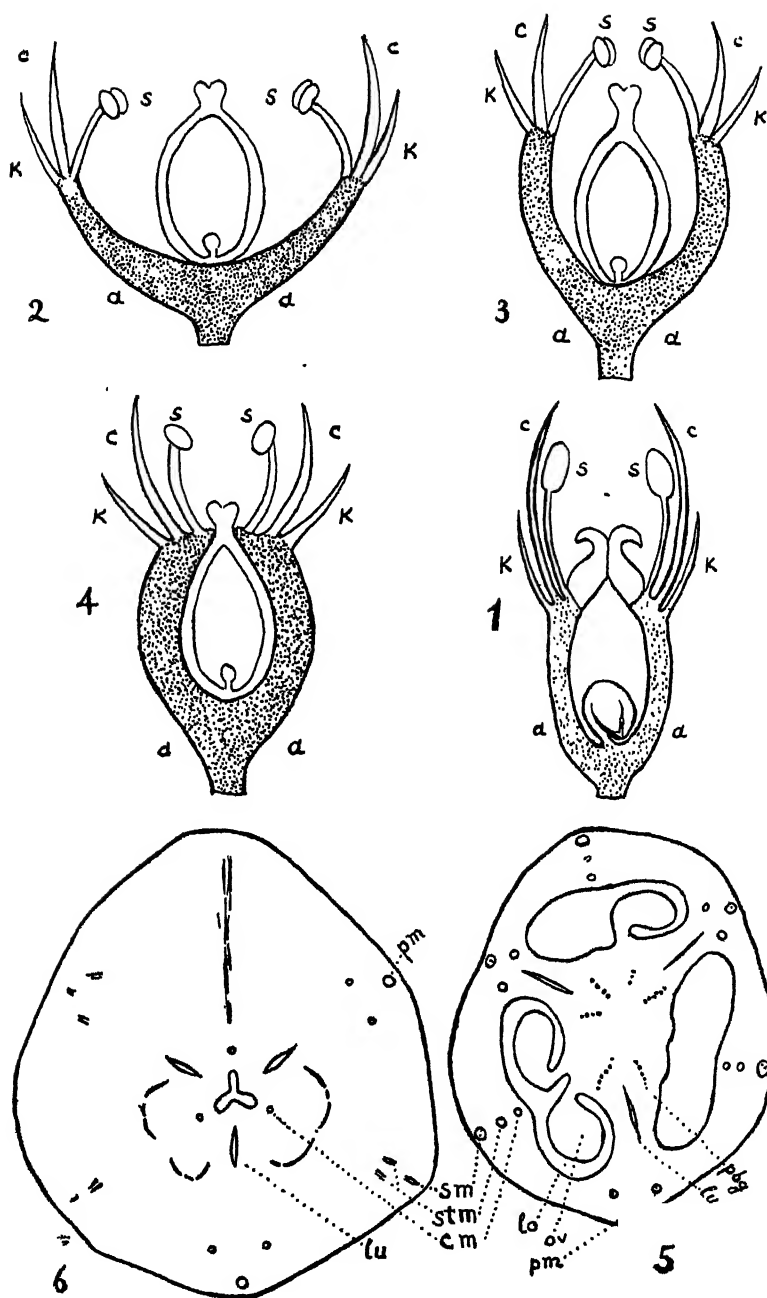
THE INFERIOR OVARY

By EDITH R. SAUNDERS

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(With 11 figures in the text)

THE view generally accepted—without exception might be ventured in the case of British and German writers—concerning the mode of formation of the inferior ovary finds illustration in almost every current text-book which makes reference to the epigynous flower. These illustrations are almost invariably based on essentially the same model. They represent the successive floral whorls as borne upon a deeply concave or cup-shaped axis from the rim of which arise in normal sequence sepals, petals and stamens. The exact mode of development of the gynoeceum, however, on this view, remains obscure, and has, in fact, never been very satisfactorily explained. The older type of figure, still however to be found in current works and here reproduced (Fig. 1) represents the carpels as also springing from this rim, and at the moment of origin they are conceived as merely roofing in the hollow axis, though the placentae eventually extend down to the base of the concavity. How it can come about that the vascular strand of the carpel midrib is prolonged upwards into the style while the placental cords are presumed to extend down to the base of the concavity is difficult to understand. A later modification of this view and the one more generally accepted (illustrated in Fig. 4—compare with Figs. 2 and 3, in which the condition of perigyny (slight in Fig. 2, more pronounced in Fig. 3) is represented for comparison) supposes the edges of the concave axis itself to meet (or almost to meet) over the fertile region of the carpels which themselves are regarded as springing, not from the rim, but from the base of the axial cavity, and as being completely



fused on their exterior with the enveloping axial tissue. This view, in fact, conceives epigyny as merely an extreme case of such a type of perigyny as is seen in *Pyrus*¹. On this interpretation the area of naked ovary will depend upon the height to which the (supposed) axis tissue extends up the outside of the gynoecium. Ordinarily this uncovered region appears to be of negligible extent but in the capsular fruits of *Alstroemeria* (Amaryllidaceae) and *Moraea* (Iridaceae) it is exceptionally large and conspicuous. This explanation, though less difficult of belief than the earlier interpretation, seems equally unfounded. Both views, in fact, appear to have arisen owing to the necessity for providing some working hypothesis rather than as the result of any definite evidence which can be adduced in their support. In no case, indeed, have I been able to discover any statement of facts in the text accompanying the above diagrams which can be said to establish the correctness of either supposition.

Now the fundamental idea underlying both these conceptions is the envelopment of the gynoecium by the floral axis as the result of arrest of the growth of the apex and continued growth of the sides in such a manner as to produce a concavity, the walls of which are presumed to be composed externally of axial and internally of carpellary tissue, the one merging imperceptibly into the other. It chanced that in the course of a recent investigation into the construction of the gynoecium in general² the ovaries of numerous hypogynous and epigynous types were examined and included among the former were certain members of the Geraniales. From this work there emerged the conception of the polymorphic carpel, and in the case of the Geraniaceae and their allies the further conclusion that

LEGEND TO TEXT-FIGURES 1-6.

FIGS. 1-6. 1. Diagram of epigynous flower (after Prantl and Vines). 2, 3. Two types of perigynous flower. 4. Epigynous flower (after Strasburger). *a*, axis, *k*, sepal, *c*, petal, *s*, stamen. 5, 6. Gynoecium of *Aristea corymbosa* Benth. and Hook. in transverse section: 5, through the ovule-bearing region; 6, near the apex (slightly oblique). In 6 the placental vascular bundles have come to an end, the loculi are becoming roofed-in and one carpel midrib is seen running in horizontally towards the centre in line with one arm of the stylar canal; the midrib bundles here resume their vertical course and pass up into the styles. *ov*, ovule; *lo*, loculus; *lu*, lumen of septal gland; *pbg*, placental bundle group; *sm*, sepal midrib; *pm*, petal midrib; *stm*, stamen midrib; *cm*, carpel midrib.

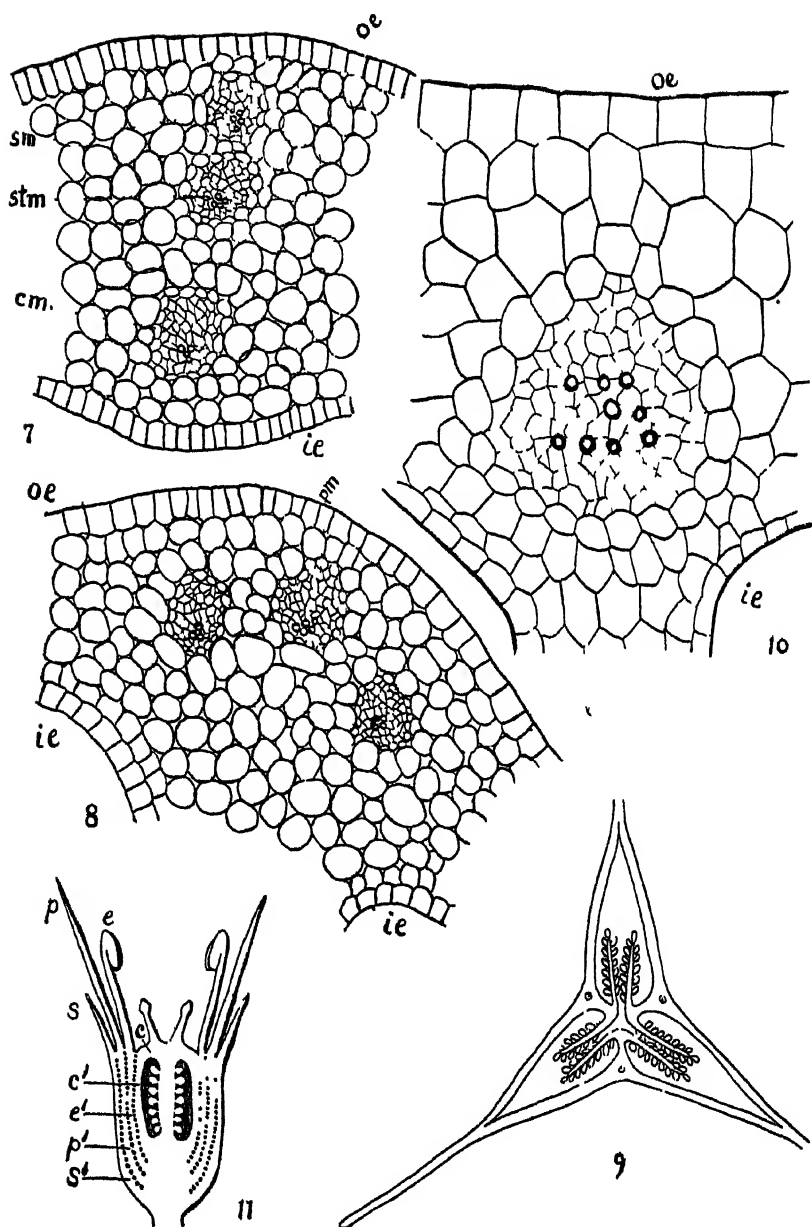
¹ Cited by some writers as an epigynous type. But even on the orthodox view the only line that can be drawn is between the condition in which even at maturity an opening remains above through which the styles (or style) project(s) (perigyny), and that in which the roofing-in of the fertile portion of the gynoecium is complete (epigyny).

² See *Annals of Botany*, 37, p. 451. July, 1923.

the current view regarding the nature of the carpophore was untenable and at variance with the leaf-skin theory of the construction of the shoot¹—a conception equally applicable to the floral as to the vegetative axis, and which, as regards the carpophore, shows the necessity for adopting a carpellary in place of an axial interpretation of this structure. The leaf-skin theory having thus proved a valuable aid to the elucidation of gynoecium structure in certain *hypogynous* types, its application to the problem of the *epigynous* flower naturally suggested itself. Now the leaf-skin theory requires, if the lower portion of the fruit wall be indeed axial in nature, that it should be clothed with the downward extension of foliar members which are exerted at a higher level. In the case of the epigynous flower such extensions must clothe the outer surface of the putative concave floral axis and must obviously be derived either from the sepals only or from both perianth whorls, and will be composed in accordance with our present knowledge of not less than two cell layers—the epidermal and the outermost cortical layer. *It follows that if in the thickness of the wall enclosing the ovary cavity there are no more than two cell layers external to the vascular cords which furnish the midribs of the perianth segments, and if the whole of these cords is used up to furnish the midribs of the various floral members, no part of this containing wall can be composed of axis tissue.* In order to investigate this point the ovaries of a number of epigynous types were examined in transverse section, including certain Iridaceous species and among them *Aristea corymbosa* Benth. and Hook. Sections taken at successive levels showed the usual type of construction (Figs. 5 and 6)—a trilocular ovary, septal glands in the partitions, vascular cords on the six radii corresponding to the perianth segments, and twin placental bundle groups at the inner end of each septum, on either side of the lumen of the gland. The vascular cord on each sepal radius gives off a vascular bundle from its inner face, and this latter strand in turn repeats the process so that the three bundles lie in radial line (Fig. 7). Upwards they diverge respectively into the sepal, stamen and carpel lying on this radius. On the three alternate radii the corresponding vascular cord provides the midrib of the petals, being flanked on either side by a smaller bundle which eventually forks to form the marginal vein of petal and sepal where the two become disjoined (Fig. 8). *On the radius of both sepals and petals the number of cell layers outside the vascular strand of the midrib is two—one epidermal and one hypodermal.* Now, as stated above, if any

¹ See *Annals of Botany*, 36, p. 135. April, 1922.

portion of the ovary wall is composed of axis tissue such tissue must necessarily lie deeper than the two outer cell layers, since these layers would represent the downward extension of the perianth members which are exerted at a higher level. But in *Aristea corymbosa* these two layers are seen to abut directly on the vascular cords which become the midribs of the perianth members. In this plant therefore it is clear that there is no space for any axial tissue and in fact that no such tissue is present. The ordinary conception of the composite nature of the wall of the inferior ovary, arising as the result of the fusion of the edges of an axial cup over sunk carpels, is thus clearly untenable in this case—a case of normal epigyny occurring in a family in which, moreover, epigyny is a typical feature. Instead of fusion throughout (or almost throughout) its whole length of the ovary with the axis we have concrescence, or, as it might be better termed, non-separation for a considerable distance of the superposed foliar members on each radius. It follows that the exertion level of sepal, petal and stamen, though seemingly the level of origin is not the real level at which they become differentiated from the axis. The fruit-wall is thus shown to be composed solely of the tissue of these foliar members which differ from those of the hypogynous flower only in this respect, that the basal portions of superposed members remain joined as far as the summit of the ovary, although the vascular cords destined for these members become separate quite near the ovary base. But seeing that the concave-floral-axis theory is thus definitely disproved in the case of *Aristea*, have we any grounds for retaining it in the case of members of the Iridaceae in which the vascular cords continued into the perianth segments happen to lie deeper than two cell layers from the surface? And if it is unfounded in the Iridaceae is it not probably an equally gratuitous assumption in the case of other families? Direct proof is only obtainable in the present state of our knowledge in cases where the vascular cords destined for the perianth are covered by not more than two cell layers of fruit wall and this type appears to be far less common than that with a more massive wall. In the Begoniaceae, however—another typically epigynous family chosen at random—further evidence confirmatory of the present standpoint was obtained. In a form of *Begonia corallina* Carr, the vascular strand running up each of the three flat sides of the triangular, 3-winged fruit and furnishing the midribs of the foliar structures (= perianth) exerted above the ovary and of the three semi-solid carpels is covered by two cell layers only, so that here also it is evident that no axial tissue can envelop the ovary (Figs. 9 and 10).



The possibility that concrescence of the different whorls might perhaps be the clue to the inferior ovary has been previously suggested by van Tieghem¹ and by Bonnier and du Sablon², who represent the epigynous condition in a diagram which shows the wall of the ovary composed exclusively of the basilar prolongations of the several floral whorls³ (see Fig. 11). This diagram is, however, as stated in the text, purely theoretical, and no evidence is advanced in its support. Van Tieghem also neither gives reasons for his view nor any facts in support of it. As we have seen, however, by applying the leaf-skin conception of the stem to the floral axis we are enabled to adduce definite proof in the cases of epigyny cited above of the *exclusively foliar* nature of the ovary wall and thus to establish these authors' supposition. This being so, it may well be questioned whether the abstract cup-shaped-floral-axis conception can any longer be retained for cases of genuine (so-called) epigyny, for which, in view of the facts detailed above, the term *syngyny* might with advantage in future be substituted. The same three types of flower would be distinguished as before, but under the terms hypogynous, perigynous and syngynous. By discarding the term epigyny we should get rid not only of the inaccuracy inherent in the word itself, but also of the assumption regarding the shape of the floral axis inevitably associated with it, which has been shown above to be unfounded.

For the drawings of the microscopic preparations and of the figures cited from other works here reproduced I am indebted to Miss D. F. M. Pertz, to whom I here tender my very grateful thanks.

LEGEND TO TEXT-FIGURES 7-11.

FIGS. 7-11. 7, 8. *Aristea corymbosa* Benth. and Hook., portion of wall of the ovary in transverse section (highly magnified), showing the epidermal and one hypodermal layer covering the vascular bundle which furnishes the midrib of the perianth members: 7, on the radius of sepal and locus; 8, on the radius of petal and septum. *oe*, outer epidermis; *ie*, inner epidermis; *sm*, sepal midrib; *pm*, petal midrib; *stm*, stamen midrib; *cm*, carpel midrib. 9, 10. *Begonia corallina* Carr. 9. Gynoecium of three semi-solid carpels in transverse section, showing the carpel midrib in the middle of each flat side. 10. Portion of the ovary wall highly magnified, showing the epidermal and one hypodermal layer outside the carpel midrib. 11. Diagram of epigynous flower (after Bonnier and du Sablon), showing the ovary wall composed of the basilar prolongations (*s'*, *p'*, *e'*, *c'*) of the sepals, petals, stamens and carpels (*s*, *p*, *e*, *c*).

¹ *Traité de Botanique*, 1, p. 402. 1891.

² *Cours de Botanique*, 2, p. 544, Fig. 866. 1902.

³ Not to be confused with the downward extensions of superficial tissue which constitute the leaf-skin.

THE EFFECT OF LIGHT OF DIFFERENT WAVE-LENGTHS ON THE RATE OF REPRODUCTION OF *VOLVOX AUREUS* AND *CLOSTERIUM ACEROSUM*

By A. BROOKER KLUGH

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(With 2 figures in the text)

A VERY extensive literature exists concerning the relation between light and photosynthesis, and this fundamental biological problem has been approached from many angles. One of the most important questions in connection with this problem is as to whether photosynthesis is a wave-length phenomenon or whether it is, at least within the limits of the visible spectrum, a matter of total light intensity only. I shall not, in this brief paper, review the literature on this point, but shall merely remark that the conclusions of different workers are diametrically opposed.

The method used in the two experiments mentioned in this paper is, I believe, quite different from any of the methods which have previously been employed, and it is for this reason, and because the results are clear-cut and striking, that this preliminary paper is presented.

This method, briefly, is to grow unicellular, or colonial, chlorophyll-containing organisms in light which is of the same intensity but of different wave-lengths, and to take their rate of reproduction as a criterion of the efficiency of the wave-lengths concerned for photosynthesis. This method may, certainly, be regarded as indirect, as other things besides photosynthesis are involved in the reproduction rate, but fundamentally the rate of reproduction in chlorophyllous organisms is obviously dependent upon their ability to manufacture food. Furthermore, experiments conducted in the same manner on two other chlorophyll-bearing organisms gave very similar results, while experiments on two non-chlorophyllous organisms yielded quite dissimilar results. These latter experiments will be dealt with in a subsequent paper.

The method of experimentation was as follows:

Three boxes were constructed, each having the front open and

having a light-tight door behind. Over the front of each box a colour-filter was fixed, these filters being so chosen that the spectrum was divided into three portions with but little overlapping. The filters selected were Wratten filters Nos. 26, 58 and 47. The red filter, No. 26, has a total transmission of 22 per cent., and a transmission greater than 10 per cent. only from $\lambda 590\mu$ downwards. The green filter, No. 58, has a total transmission of 23 per cent., and a transmission above 10 per cent. only from $\lambda 580$ to $\lambda 490$. The blue

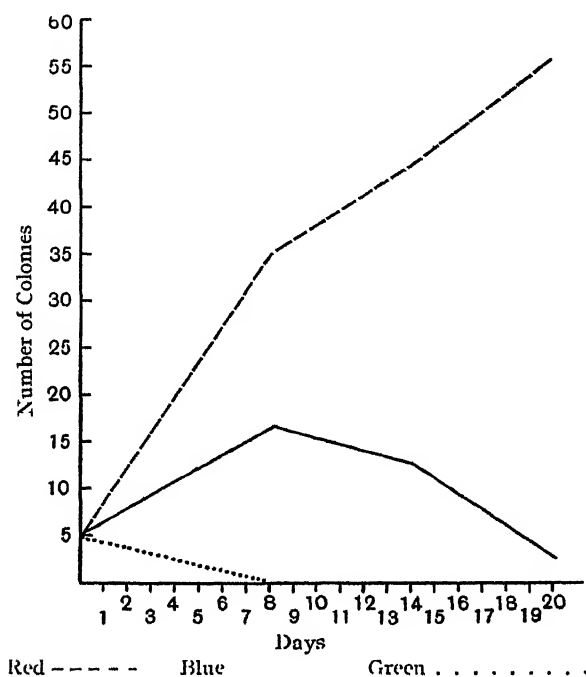


Fig. 1. Reproduction-rate of *Volvox aureus*.

filter, No. 47, has a total transmission of 2.9 per cent., and a transmission greater than 10 per cent. only from $\lambda 500$ upwards. The spectrophotometric curves of, and full transmission data on, these filters are given in *Wratten Light Filters* issued by the Eastman Kodak Company. All the filters were brought to very nearly the same total transmission value (*i.e.* a close approximation to 2.9 per cent.) by placing a prepared film filter of approximately 12.5 per cent. behind the red and green filters in the "colour-boxes." These neutral film filters were prepared in the following manner. Pieces of

photographic film were exposed to a weak light for various brief periods, then developed, fixed and dried, and matched, visually, against a set of neutral filters of known percentage transmission. Two of the prepared filters which came, as nearly as could be judged,

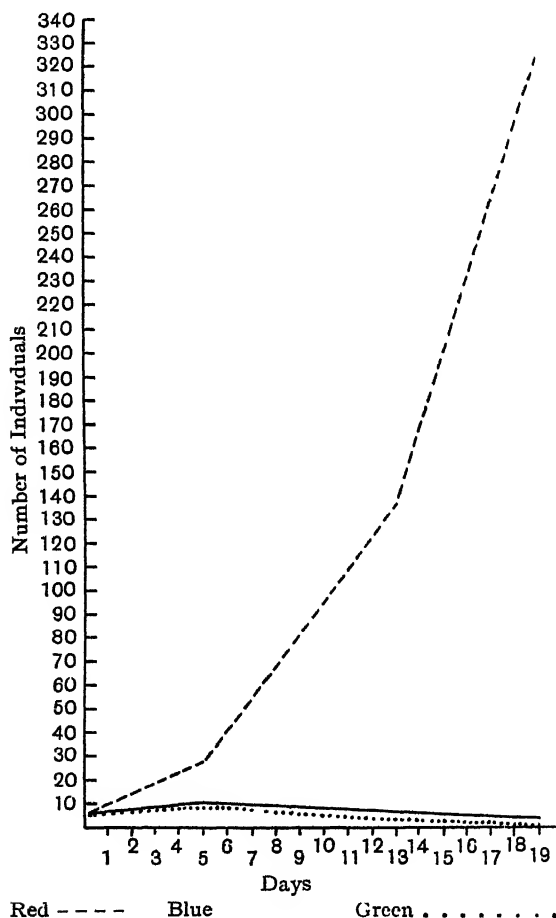


Fig 2. Reproduction-rate of *Closterium acerosum*.

midway between the 10 per cent. and the 15 per cent. filters of the set of known transmission were selected. This method of obtaining neutral transmission filters is, of course, by no means as accurate as the use of a standard photometric instrument, and in future work it is planned to use only accurately calibrated filters, but never-

theless, the neutral filters used in these experiments, while they may not have been exactly 12.5 per cent., were certainly between 11 and 14 per cent. transmission.

The boxes, with the filters in position, were set up side by side in an east window, where they received two hours' sunlight on sunny days. Five individuals or colonies were placed in each of three vials, in filtered water from the habitat of the organism, and a vial placed in each box. The number of the organisms present in each vial was recorded at intervals of several days. Constant checking with a thermometer showed that there was no difference in the temperature of the three boxes.

Experiment on *Volvox aureus*.

Five colonies of approximately similar size were placed in each of the boxes on August 8th, and the readings were as follows:

	Red	Green	Blue
Aug. 16	35	0	16
„ 22	44	0	13
„ 27	56	0	3

These results are shown graphically in Fig. 1.

Experiment on *Closterium acerosum*.

Five individuals of this species were placed in each box on Aug. 22nd, and the readings were as follows:

	Red	Green	Blue
Aug. 24	17	6	6
„ 27	27	9	10
Sept. 4	138	4	5
„ 10	324	1	4

The results of this experiment are shown graphically in Fig. 2.

CONCLUSIONS

It is dangerous to draw conclusions from a single series of experiments, even when the results are perfectly clear-cut. But we can at least say that these experiments indicate that the wave length of the light plays a very important part in the metabolism of these two organisms, and that, if reproduction rate is a criterion of photosynthetic activity, photosynthesis is a wave-length phenomenon, red light being highly efficient, blue much less so, and green inefficient. The slight increase in the numbers of *Closterium acerosum*, at first, in the green, may possibly be explained by the fact that some at

least of the individual desmids had a sufficient store of food to enable them to divide. Several other matters, some of them rather complex, in regard to light as a biotic factor, suggest themselves in connection with these experiments, but a discussion of them must be left until further experiments provide adequate data for their elucidation.

SUMMARY

Five colonies of *Volvox aureus* were placed respectively in red, green and blue light and their rate of reproduction was ascertained; and five individuals of *Closterium acerosum* were treated in the same way. If the rate of reproduction be taken as a criterion of photosynthetic activity then photosynthesis is a wave-length phenomenon, red being most efficient, blue much less so, and green inefficient.

THE IMPORTANCE OF ABSTRACTING OLD BOTANICAL WORKS AND MANUSCRIPTS

THE value of a complete abstract journal cannot be over-estimated; we all recognise the great importance of periodicals such as *Botanical Abstracts*, *Botanisches Centralblatt*, *Chemical Abstracts* and a number of similar papers in other fields of learning.

These publications, however, are of relatively recent origin. For example, Just's *Botanische Jahresbericht* was founded by Just in 1873. *Botanisches Centralblatt* was started in 1880.

Previous to this time a wealth of important work had been published, some of which can be found in a number of well-known serials. Some of them are still being published, but the earlier issues are not easily obtainable, except in some of the larger institutions of the Old and New World. Of these publications we need mention only *Curtis's Botanical Magazine* (1786), *Botanische Zeitung* (1843), *Linnaea* (1826), *La Belgique Horticole* (1851), *Transactions and Proceedings of the Botanical Society of Edinburgh* (1843), *Transactions of the Royal Society* (1663), *Flore des Serres et des Jardins de l'Europe* (1845), and a number of others.

I have spent considerable time in a number of libraries like those of the Royal Botanic Gardens, Kew, of the British Museum, of the University of Amsterdam, of the University of Berlin, and others, studying works on plants and on their economical aspects published

in earlier centuries. One is surprised by the fine talent of these observers and workers of times long past, men who were heretofore unknown to us. From the sixteenth until the beginning of the last century men like Malpighi, Grew, Swammerdam, Leeuwenhoek, Camerarius, Linné, Koelreuter or Sprengel have given us an indelible impression of their achievements, but there are others whose work has been forgotten, and no one can estimate the advantage it will be to science and history to have their work revived. If we consider only the work of an investigator of later date, namely, that of Gregor Mendel, one will realise the importance of this task of abstracting.

There are international catalogues besides those of libraries which will facilitate this work considerably. Also publications like Seguiet's *Bibliotheca Botanica*, 1740, Wikström's *Litteraturæ Botanica in Suecia*, 1831, M. S. Krüger's *Bibliographia Botanica*, 1841, and treatises on the history of botany during certain periods which will contribute very much toward making the task of finding originals easier.

In many libraries there are unknown manuscripts, which are not published and probably never will be published, but which are of enormous value. They may contain views or observations which were not ripe for those days, and therefore were not fully comprehended by the learned world of that time. The work of the abstractor, especially in abstracting manuscripts will become very difficult, and here the co-operation of various institutions will be of the greatest value.

No doubt everybody will agree as to the necessity of this work, but it is difficult to state to what extent it should be done, whether the purely botanical side should end with the beginning of the publication of Just's *Botanische Jahresbericht* or with the economical aspects of botany, for example, with the foundation of Experiment Station Record, or something else, is difficult to suggest.

As far as the bulk of abstracting is concerned up to the beginning of the nineteenth century it will not be as great as the work accomplished during the last three to five years.

A systematic search, a perseverance and last, but not least, a love for one's profession, will ensure the completion of this work, which will not only be of great historical value, but also of inestimable value to science itself.

J. C. TH. UPHOF.

REVIEW

MALAY FUNGI.

Icones Fungorum Malayensium. Abbildungen und Beschreibungen der malayischen Pilze. Herausgegeben von Dr C. VAN OVEREEM und Prof. Dr J. WEISE. Published by Martinus Nijhoff, The Hague, 1.50 guilders per part.

This work, of which Hefte 1-iv have so far been published, is intended to comprise a complete account, with illustrations, of all the fungi found in the Dutch East Indies. The first four parts deal with the Clavariaceae, in which members of the genera *Clavaria*, *Clavariella*, *Clavulina*, *Clavulinopsis*, and *Phaeoclavulina* are described. No particular order is followed in the sequence of species. The full diagnoses in German are accompanied by measurements of the spore-producing organs and spores. The coloured plates of the fungi are particularly good, and the basidia and spores are shown on an adequate scale. Most of these fungi are exclusively tropical forms, but some are of cosmopolitan distribution such as *Clavaria vermicularis*. There are few tropical countries of the world in which the fungus flora has been explored at all carefully, and this is one of the first attempts to describe fully the fungi of a tropical region. These *Icones* should be of great service to other workers in tropical mycology.

F. T. B.

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A NOTE ON THE OCCURRENCE OF NATURAL PRESERVATION OF PLANT TISSUES¹

By

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(With Plates V and VI and 5 figures in the text)

THE present note refers to the discovery of certain stems and roots of halophytes in a remarkable state of preservation, non-petrified, in an outcrop of marsh mud on the beach at Blakeney Point, Norfolk, and also to some investigations of pans on the salt marsh undertaken with a view to finding out the occurrence of conditions suitable to preservation.

TOPOGRAPHY OF BLAKENEY POINT

Blakeney Point consists of a long shingle spit (Map, fig. 1), the broader distal portion of which is known as the Headland and bears an extensive sand-dune system. The shingle spit, itself a straight run of 7 miles, connects the Headland to the mainland and is now entirely destitute of a sand-dune covering, being just a bank of loose sand and shingle.

The spit runs roughly east and west. Its southern face abuts on the salt marshes developed in Blakeney Harbour, while its northern face is open to the sea.

The topography need not be described in further detail here, as this has already been done elsewhere (1).

During exceptionally high tides, such as occur from time to time, especially in winter, when there are on-shore gales, the loose shingle on the seaward face of the spit is carried up over the crest and is deposited as talus fans upon the marsh (1,4). A photograph of such fans projecting into the marsh is shown in Plate VI, fig. 1.

¹ Blakeney Point Publication, No 24

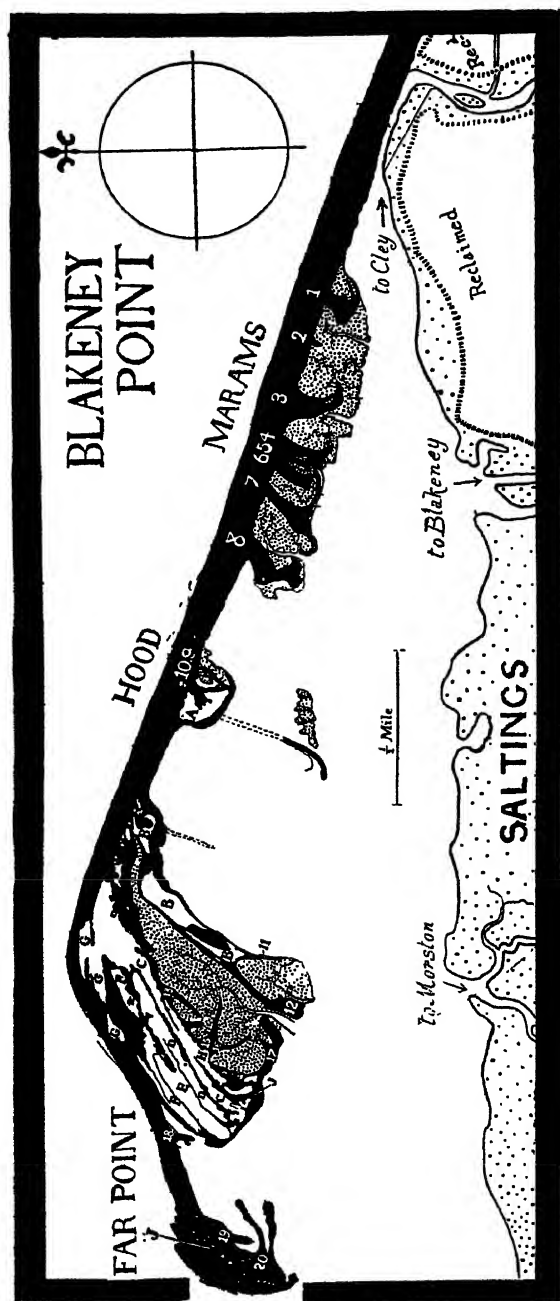


Fig. 1. Sketch-map of Blakeney Point. Exposed shingle, black; salt marshes, dotted; sand dunes, channels and bare muds, white. The lateral shingle beaches are numbered in their order of development, whilst the successive systems of dunes are lettered A to J. The outcrop occurred on the sea face of the main beach between the figures 1 and 2. Approx. scale, $1\frac{1}{4}$ inches to mile.

In this way, the shingle beach is gradually moving southwards over the marsh, while the buried marsh surface tends to outcrop on the seaward side of the spit.

Usually the marsh outcrop is not visible, being buried beneath 4-5 feet of shingle, its position being indicated at low water by springs which rise along the beach at about the same level.

Under certain conditions of wind and tide the covering of shingle may be removed, exposing the old marsh mud, a rare event. This was found to have occurred in September 1923 during the visit to the Point of a working-party from University College, London, and furnished the opportunity for examining the outcrop which concerns the present note. Another exposure of the same kind is figured in Carey and Oliver (1), Plate XXVII.

THE OUTCROP

The outcrop was situated on the Marams, between the numbers 1 and 2 on the Sketch-map (Fig. 1). It extended along the beach for a distance of about 100 feet, and had been weathered to the beach angle (Plate V, fig. 1). It consisted of a brown humic clay 3 feet 5 inches thick overlying a zone of blue clay 1 foot 9 inches thick, underneath which was sand.

Pits were dug in the outcrop and also in the marsh on the opposite side of the shingle spit. Levellings were also made across the spit and the results are incorporated in Fig. 2 below. This shows a profile of marsh, spit, and outcrop, with vertical factor $\times 5$.

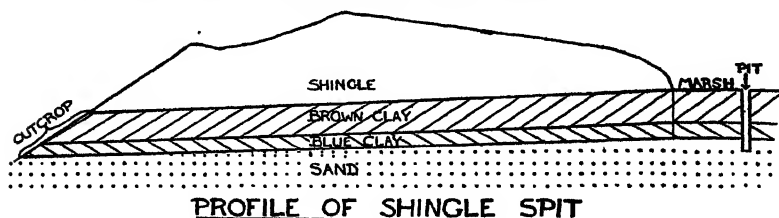


Fig. 2. Profile of shingle spit at the Marams, vertical factor $\times 5$. The outcrop appears on the left-hand side of the figure, the beach, and on the right a portion of the marsh showing the pit dug out to examine the strata. The vertical depth of the brown clay at the outcrop was 3.4 feet and 3.5 feet in the marsh opposite the outcrop, whilst for the blue clay the corresponding figures are 1.8 feet and 1.9 feet. The drop in level of the upper limit of the brown clay passing from marsh to beach amounts to 2.5 feet. The width of the covered belt of clay in contact with the shingle is 274 feet.

From this profile it will be seen that the correlation of strata on both sides of the spit is good, and justifies the assumption that they

are continuous from one side to the other. The dipping down of the strata from marsh to outcrop is probably due, in part, to the pressure of overlying shingle, and in part to the fact that once buried under the shingle, accretion ceases, while the normal marsh surface goes on rising.

The thickness of the strata, brown and blue clays, at the outcrop is also about 3 inches less than that found on the marsh side which would be accounted for by the cessation of accretion.

THE PLANT REMAINS

The plant remains were found in the upper regions of the outcrop, the top 2 feet, roughly speaking. Towards the base of the brown clay there was no good preservation, though the clay was well supplied with humic material. There were no plant remains in the blue clay, nor recognizable humus particles. The blue colour of the clay may have been due in part to organic matter in a very finely divided state.

The remains consisted mainly of stems and roots of *Statice Limonium* preserved *in situ*. A few stems of *Obione portulacoides* were also found, easily recognizable by their swollen nodes and long internodes. In washing out some of the *Statice* stems from the clay, a few seeds were also found. They appeared to be confined to a narrow stratum only about 5 mm. thick. It is difficult to identify these seeds with any great degree of certainty, but by comparison with a collection made on the Point they would appear to be the seeds of an *Atriplex*, and probably *A. patula*. At the present time this *Atriplex* has only a limited distribution at Blakeney, about a mile from the outcrop. Three of these seeds are shown in Plate V, fig. 3. One of them was just germinating at the time it was buried. Unfortunately, the radicle became detached from the seed during mounting.

The *Statice* stems occurred in large numbers so as to be quite conspicuous, jutting out from the weathered surface of the outcrop. They were in a blackened, carbonaceous condition, and rather brittle. Leaves had all disappeared and the tips of the stems had been worn off by wave action at high tide. When removed from the clay, only traces of the fibrous roots were found. The larger roots and stems had, however, undergone very little decomposition or change in external appearance as compared with living plants from the marsh.

In some places casts of a hard ferruginous material were found. They were hollow in the centre and may have been formed around nuclei of *Statice* stems which had afterwards entirely decayed, or they may have been the holes of marine worms which occur in the living condition in pans on the salt marsh.

The preserved material was removed in slabs of clay to the Laboratory for further examination, where it was carefully washed out of the clay.

It was found that the stems and roots could be cut quite easily with a razor. Owing to the brittle character of the material, hand sections so cut were necessarily somewhat thick but served quite well for a preliminary examination.

None of the stems or roots exhibited the slightest traces of petrification, they were simply in a blackened carbonized condition. For this reason they have been referred to here as "preserved material" in preference to employing the word "fossil," which is commonly associated with petrification.

After embedding in paraffin the material was sectioned on a Minot microtome. Some difficulty was experienced in mounting sections cut at 8μ owing to their delicate nature. However, it was found satisfactory if they were first placed in very dilute Canada balsam in xylol and this allowed to concentrate before mounting in normal strength.

A microscopical examination of the sections revealed the structure typical of *Statice Limonium*. They were readily identified by comparison with sections of living *S. Limonium* plants from the marsh. The well-developed periderm was still intact. It is more pronounced in the stem than in the root. The spongy lacunar cortex was also well preserved. A very characteristic feature of the "preserved material," and one which confirmed the identification, was the presence in the cortex of nests of sclerotic elements which occurred in many of the specimens examined. Plate VI, fig. 3, is a photo of a "preserved" stem in transverse section, and shows a prominent group of sclerotic cells. These cells occur in the stem of *S. Limonium*, but are not present in the root. Such groups of sclerotic cells are to be found in other species of *Statice* which grow at Blakeney and have been described by De Fraine(?); but these other species do not produce stems so large as those found in the outcrop, which had diameters up to 1.2 cm.

PROBABLE AGE OF THE "PRESERVED MATERIAL"

As has already been stated, the shingle spit is travelling laterally across the salt marsh and at a rate of roughly 2 feet per annum, a determination based on direct observation of the lee fringe, and also of the movement of the crest of the spit in relation to a fixed line of telephone poles. Assuming that the rate of travel, determined from

observations during the period 1912-1924, is comparable to that of much longer periods, the spit must have taken 150-170 years to travel its own width (1). This, therefore, is the minimum time that the material has lain buried in the marsh mud. To this period must be added a further term of years, as it is evident from the existence of "preserved material," down to a depth of about 2 feet from the upper limit of the outcrop, that this material must have been already buried and under conditions of preservation before the advance of the shingle spit had reached that part of the marsh. If the preserved material had been derived from plants which were living upon the marsh up to the time of the arrival of the shingle, it would only have occupied a narrow zone at the junction of outcrop and overlying shingle.

In all probability, therefore, the age of the preserved material will be more accurately estimated at upwards of 200 years.

THE PROBABLE CONDITIONS OF PRESERVATION

It was noticed that whereas the outcrop contained abundant plant remains, the corresponding stratum on the turf-covered marsh showed no preservation, the clay containing only humus particles. This suggests that conditions favourable to the burying and preservation of plant stems and roots occurred only in certain specialized areas. The outstanding areas which differ greatly from the marsh as a whole are the pans. These are areas of varying form and size, mainly bare mud with the surface a few inches below the general level of the salt marsh turf.

It was hoped that it would be possible to re-examine the outcrop in July 1924, when a party was again visiting the Point, with a view to comparing the outcrop clay with pan clay, to determine how far they resembled each other, and also to determine the exact distribution of preserved material in the outcrop. At this time, however, the outcrop was buried beneath $4\frac{1}{2}$ feet of loose shingle. It was located, but it proved impracticable to clear out a hole large enough to work at it before the next tide washed the shingle back again into the digging. Further work was therefore confined to an examination of pans on the salt marsh itself, as it seemed possible that they might have provided conditions suitable to preservation.

THE PANS OF THE SALT MARSH

Two extreme types of pan were found in the marshes at Blakeney Point, and in the Stiffkey marshes which are across the harbour, due south of the Far Point (Sketch-map, Fig. 1, p. 194).

For convenience, these types may be termed "Hard" and "Soft" pans, according to the nature of their floors. This distinction is not an absolute one as pans of an intermediate character do occur, but apparently not in great numbers.

In hard pans the mud in their bottoms is only about 6 inches thick, overlying sand. During the period of neap tides, when the marsh is not covered, these pans dry out completely. The mud in them hardens and cracks, allowing aeration to take place. The mud is, in consequence, of a grey colour, and apparently does not contain very large amounts of organic matter.

Soft pans have floors of very fine black mud and clay, which may be many feet deep. They retain water from one period of spring tides until the next, and, except for certain corners which are shallower, the mud never dries and cracks.

In no instance was any "preserved material" found in the comparatively well aerated mud of hard pans.

In a pan of intermediate character, located in the third marsh from the Watch-house of the Marams system (opposite number 6 on the Sketch-map, Fig. 1), stools of dead *Statice Limonium* plants *in situ* were found in abundance, fringing the edge of the pan and extending over the floor of the pan for some 1 to 2 feet from the margin (Plate V, fig. 2). Plants of *Obione portulacoides* were also dying off and their stems falling into the mud.

The *Statice* stools were not so far advanced in decomposition as those from the outcrop, being much less brittle. Externally the material from the two sources was almost identical. Plate VI, fig. 2, shows outcrop (O) and pan (P) material, side by side.

The mud in this pan was 9-12 inches deep overlying humic sand. The pan usually retains some water at all times, but it has been known to dry out completely in dry summers. It differs from the normal soft pan in having a much thinner deposit of mud on its floor and also in having gently sloping sides. The sides of soft pans are usually perpendicular and often undercut. It seems possible that as successive spring tides deposit more mud in the bottom of a hard pan its floor may become more impervious so that in time it would become a soft pan holding water from one period of spring tides until the next. This acquired permanence of water would be likely to kill off vegetation around the pan and may account for the dead stools of *Statice* in the pan in question. It is unknown how long the dead stools have been in this pan, but decomposition is probably slow in pan water. The concentration of salt in pans is known to be greater than that

of the sea water under certain conditions(3), and the same probably applies here.

Continued accretion, if fast enough, would bury the dead stools before they had rotted, possibly leading to the preservation of material as found in the outcrop.

A soft pan was next selected for examination, to find out if there was any evidence that soft pans are derived from hard pans by the accretion of mud in their bottoms.

This pan was located almost opposite the outcrop (between the numbers 1 and 2, Sketch-map, Fig. 1). The pan was drained with the intention of cutting a trench through it. This work was hampered by several heavy falls of rain and it was only possible to cut a comparatively shallow trench into the pan itself as the mud was exceedingly soft and continually slipped in from the sides. (Plate V, fig. 4, shows this pan after drainage.)

While the pan was draining, trenches were dug out from the edge of it. Fig. 3 is a diagram of the pan showing the trenches which were dug out.

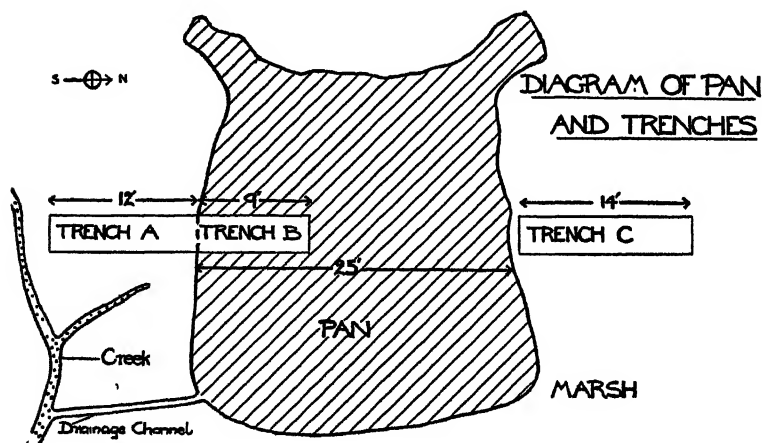


Fig. 3. Plan of the soft pan located on the Marams between the numbers 1 and 2, Sketch-map and Fig. 1, showing the trenches which were dug out to examine its structures (Figs. 4 and 5).

Trench C (Fig. 4) showed that humic sand occurred here at 7 feet from the marsh surface. It was found at 5.4 feet in the pit dug opposite the outcrop (Fig. 1) which is near by and shows that there may be considerable variation as regards the depth of the marsh mud.

Plant remains were present in a fairly advanced state of decomposition and were for the most part quite unrecognizable except close to the marsh surface. A living plant of *Triglochin maritimum* was found, the stem of which could be traced down from the marsh level to a depth of 6 inches.

A few inches behind the living crown of the plant this stem was quite dead and semi-decomposed. The elongation of the stem had apparently kept time with accretion of the marsh. Data of accretion on the marshes exist in the Blakeney Point records, and the rise on a high marsh is approximately $\frac{1}{8}$ inch per annum, so that this plant of *Triglochin* was, perhaps, 36 years old.

It is just possible that preservation does take place by the progressive burying of the older parts of the marsh halophytes, while the living crown keeps pace with the rise in level; however, from the evidence of well-decomposed plant bodies found in trench C this would not seem to be very probable.

At 4 feet 7 inches from the surface a number of bivalve shells were found, apparently *Scrobicularia piperata*, and this seems to indicate that a creek, which is now some yards away, has moved its position in course of time. Both pans and creeks are known to move.

Perhaps the most interesting fact revealed by digging trench C is the great depth, 4 feet 9 inches, to which living roots of salt marsh plants go down.

Trench A, so far as it went, was essentially similar to trench C. It was not over $2\frac{1}{2}$ feet deep.

When the pan was sufficiently drained, trench A was continued into the pan as trench B. Fig. 5 shows the arrangement of strata found, vertical factor $\times 2$. There was a well-marked layer varying from 2 to 4 inches in thickness which dipped downwards from the edge towards the centre of the pan finally reaching a fairly constant depth of 1 foot 8 inches from the surface of the pan floor. Towards the edge of the pan this layer contained well-preserved fragments of stems of *Statice Limonium*, from short bits up to pieces 2 inches long.

As the layer reached the centre of the pan, the bits of stem were gradually and completely replaced by small snail shells, very similar to those now found drifted in vast numbers in the angle facing south-west between the Hood and shingle spit and scattered over the young Hood marshes (Sketch-map, Fig. 1). These marshes have not yet reached a sufficient elevation to be colonized by *Statice*, *Triglochin*, *Obione*, etc.

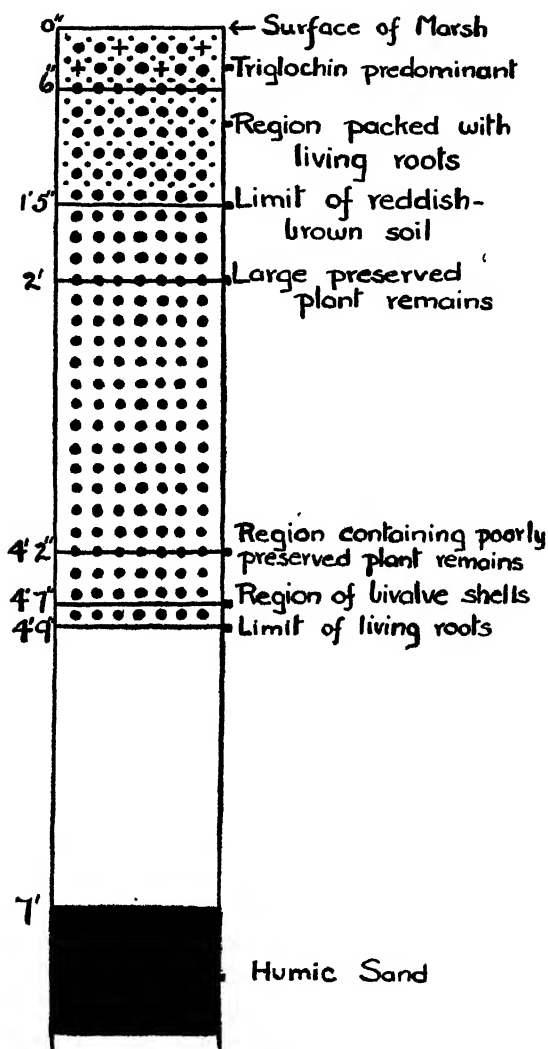


DIAGRAM OF TRENCH C VERTICAL SECTION

Fig. 4. Diagram of the strata encountered in excavating trench C. The strata were somewhat indefinite and did not lend themselves readily to a diagrammatic representation; mean values have been taken, hence the figure is not to be interpreted too rigidly.

The layer may have been formed at the edge of the pan, which, as the marsh level rose, gradually increased its area. It may be conjectured that the pan started as a soft pan on low marsh such as exists at the present time off the Hood. Small snail shells were deposited over its floor, and as accretion proceeded, around its edge also, in diminishing numbers. When the marsh was high enough for *Statice* to colonize it, the supply of shells was practically cut off and fragments of *Statice* now formed the layer, and have continued to do so up to the present time.

Above the layer of preserved *Statice* and shells the mud was very fine in texture, and black. After a few hours' exposure, however,

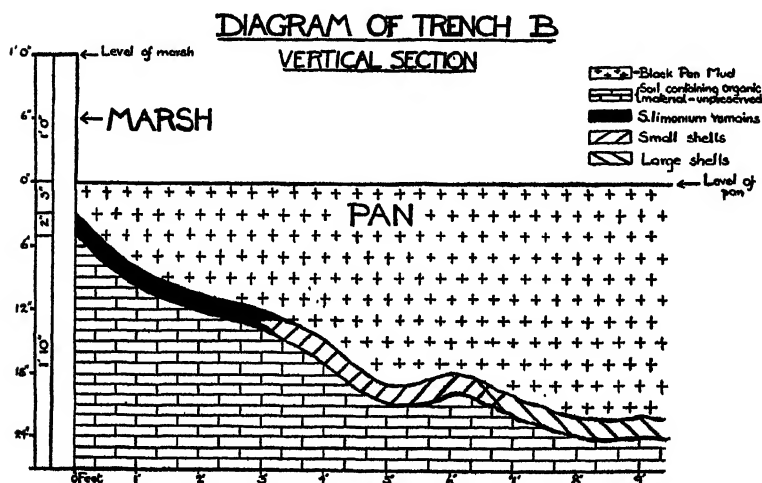


Fig. 5. Diagram of the strata of the soft pan as found by excavating trench B.

exposed surfaces had turned to a grey colour, due, apparently, to oxidation of organic matter. In this mud live numerous marine worms and bivalves (*Scrobicularia piperata*).

Below the preserved layer there was less organic matter, the mud being replaced by a bluish clay.

This pan has apparently never passed through a phase similar to that now obtaining in the pan previously mentioned. It seems to have been a "soft" pan from an early phase of the development of the salt marsh. The question, therefore, as to whether "soft" pans may be derived from "hard" ones remains unanswered.

A brief examination was made of other pans on the Stiffkey marshes. Time did not permit of systematic work, but hard and soft

pans were again found and in most soft pans, examined at random, there were abundant remains—inflorescences of *Statice* and leaves of *Obione* especially.

It should be noted that whilst the remains found preserved in the mud of typical soft pans were all drifted fragments, the outcrop contained whole plants *in situ*.

It seemed worth while publishing the results of these investigations, incomplete as they are at present, owing to the great interest attaching to problems of fossilization.

True petrification, that is, the replacement of the organic body, particle by particle, by some infiltrating agent, usually calcite, silica, pyrites or siderite, must of necessity be a very slow process. The first requirement of petrification must be that the material to be infiltrated shall be under conditions which retard very greatly or prevent decay.

There is also the problem of the types of plants which are most likely to fulfil the requirements of petrification.

At present, little evidence has been published concerning the localities in which preservation is most likely to occur, whether it be in deserts or, perhaps, in salt marshes; or if the chances are equal in all regions¹.

The Blakeney remains show how certain types of plants, some of those of the salt marsh, may be preserved for long periods with very little or no decomposition.

It was thought that, perhaps, the present note might help to further a little the investigation of the problems of fossilization.

In conclusion, the authors would like to express their gratitude to Prof. F. W. Oliver, to whom they are indebted for much practical help, suggestions, and every encouragement. They would also like to thank Mr E. M. Cutting and Mr T. G. Hill for valuable criticism in the matter of illustrations.

SUMMARY AND CONCLUSIONS

1. Well-preserved and non-petrified ("mummified") plant remains *in situ* have been found in an outcrop of marsh mud on the beach at Blakeney Point, Norfolk. They have remained buried with little change for probably upwards of 200 years.

¹ Dr Scott has very kindly drawn the authors' attention to the discovery by Bommer in the Wealden of Belgium of comparable material preserved in a carbonaceous condition, which could be cut with a razor. Though the discovery dates back some 25 years, we are not aware that the circumstances have been described in any publication.

2. The plants preserved were mostly *Statice Limonium*. A few stems of *Obione portulacoides*, and a few seeds, probably of *Atriplex patula*, were also found.

3. The conditions leading to preservation at Blakeney Point seem to be present in certain types of pans on the salt marsh, more especially the type termed "soft" pans.

4. In Blakeney Harbour, the pans of the salt marshes appear to be mainly of two types, for convenience distinguished as "hard" and "soft" pans. "Hard" pans have floors of mud only up to about 6 inches in depth overlying sand. They are filled with water when the spring tides cover the marsh, but dry out completely during the neap tides.

"Soft" pans have deep mud and clay floors and retain water from one period of spring tides until the next.

5. The type of pan is apparently primarily determined by the greater or less depth from the marsh surface at which a stratum of sand occurs. This does not preclude the possibility that by continued accretion a "hard" pan may pass over into the condition of a "soft" pan.

6. It is hoped later to elucidate more precisely by experimental work the nature of the conditions which promote the type of preservation here described.

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EXPLANATION OF THE PLATES

PLATE V

Fig. 1. General view of the beach showing the outcrop (figure standing in centre of it), looking west. The open sea on the right. The marshes are over the crest of the beach on the left. Photograph (Sept. 1923) by Prof. F. W. Oliver.

Fig. 2. Photograph of the margin of the pan opposite the number 6 (Sketch-map, Fig. 1), which is intermediate in character between the "hard" and "soft" types. On the right living plants of *Statice Limonium*, and elsewhere dead stools of the same are abundant. The darker patches on the mud are composed of prostrate plants of *Pelvetia canaliculata*. This photograph was taken towards the end of a period of neap tides and the pan had lost much of its water, the edge of which appears in the top right-hand corner (July 1924).

Fig. 3. Photomicrograph of some seeds found in the outcrop. The one at the top of the figure had begun to germinate when it was buried. The radicle

was unfortunately slightly displaced whilst mounting. Probably the seeds are those of *Atriplex patula*. $\times 11$.

Fig. 4. The soft pan, located on the Marams between numbers 1 and 2 (Sketch-map and Fig. 1), after the water had been drained off. This photograph was taken looking southwards across the marshes. Blakeney Harbour appears as a light strip running across the photo, with the mainland in the background. Plants of *Statice Limonium* and *Triglochin maritimum* are much in evidence fringing the pan (July 1924).

PLATE VI

Fig. 1. The south side of the shingle spit looking west. The loose shingle fans are seen where the spit is advancing southwards, *i.e.* from right to left, across the marsh. The dark line winding across the photograph gives the course of a creek. The pan figured in Plate V, fig. 4, can just be distinguished as a dark line on the extreme left just beyond the creek. Photograph by Prof. F. W. Oliver (September 1923).

Fig. 2. Photograph of *Statice Limonium*, crown and main root, from the outcrop (O), and portion of a dead stool, as in Plate V, fig. 2 (P). About half nat. size. The photograph gives a good idea of the remarkable state of preservation of the outcrop material.

Fig. 3. Photomicrograph of part of a transverse section of outcrop material of *Statice Limonium*. The nest of sclerotic elements in the centre of the photograph is exceptionally well preserved. The cortex is really better preserved than the photograph at first suggests, as in *Statice Limonium* it is naturally loosely organized, and alveolar. Vascular tissue on right. $\times 90$.

PERIGYNY AND CARPEL POLYMORPHISM IN SOME ROSACEAE

By EDITH R. SAUNDERS

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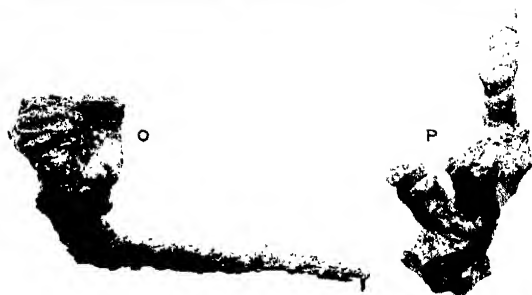
(With 33 figures in the text)

IN a recent contribution on the subject of the epigynous flower¹ evidence was brought forward showing that the accepted view that typical epigyny is to be considered as an exaggerated condition of perigyny cannot be upheld in the cases there treated. It appears, in fact, if we may generalise from these cases taken at random, that in true epigyny the concave-floral-axis conception is a wholly gratuitous assumption adopted in the past in order to provide a working hypothesis, but that it is unsupported by any evidence. Such evidence as is available shows that what occurs in epigyny is concrescence of the basal portions of the several floral whorls, which together make up the ovary wall. *Consequently the ovary wall must be considered as*

¹ *The New Phytologist*, 24, p. 179, 1925.



HUNTER AND MOTTRAM—NATURAL PRESERVATION OF PLANT TISSUES



entirely foliar in character. In the course of observations made with the object of comparing what now seems preferably termed the syngynous (otherwise epigynous) condition with various degrees of perigyny *Pyrus communis* L. was one of the latter class purposely selected for examination. For the reason that although differing from true syngynous types in not having the ovule-bearing region of the gynœcium completely roofed in, this flower has, notwithstanding, been selected, no doubt on account of its familiarity and considerable size, for description and illustration in a number of text-books as an example of *epigyny*. As, however, it is clear that on the orthodox, no less than on the present view *Pyrus* must be classed as a true *perigynous* type, and as, furthermore, the present observations have brought to light facts regarding the structure of its gynœcium which go to show that the accepted view that $G = 5$ is incorrect, the true formula being $G5 + 5$ —a construction which at once explains certain structural anomalies—it seemed desirable to employ this species for the exemplification of certain characteristic *perigynous* features as well as for the further elucidation of this type of Rosaceous gynœcium. The points of importance in this connection will be most readily appreciated from a comparison of the appearances exhibited in transverse sections taken at successive levels up the floral axis.

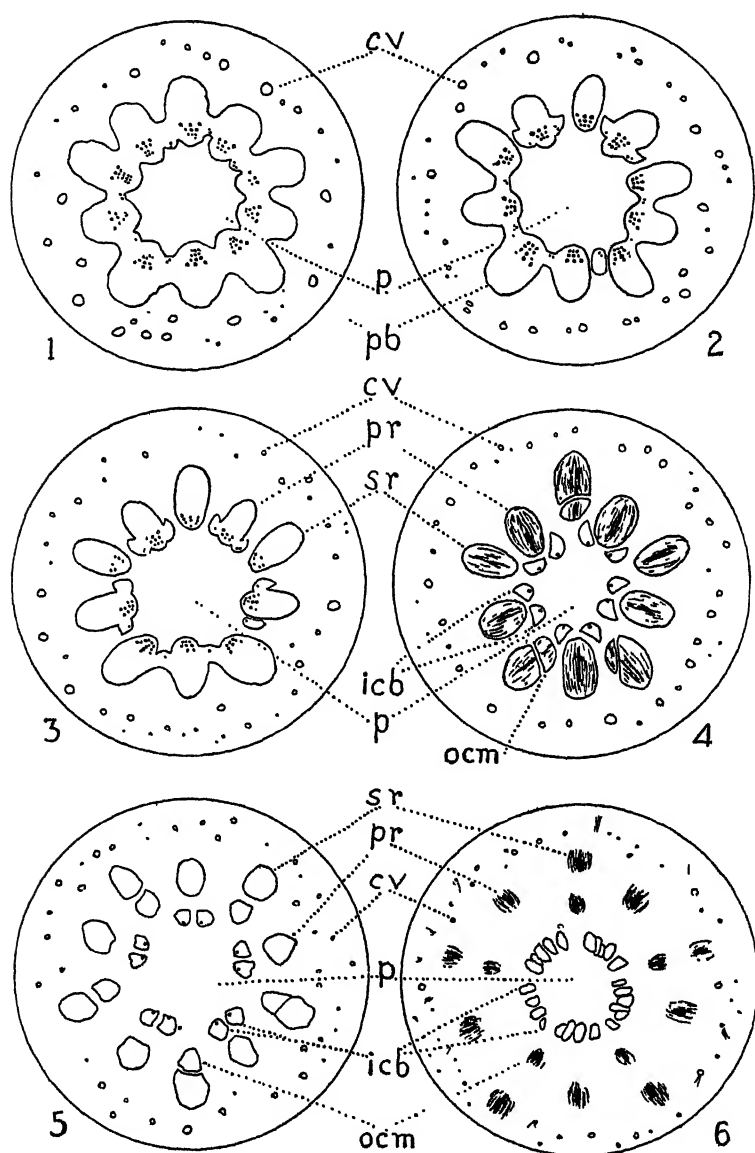
Pyrus communis L. (Figs. 1-14)

Up to a level immediately below the enlargement which carries on its free border calyx and corolla the flower stalk shows typically a ring of ten main fibro-vascular masses (primary bundles) (Fig. 1). These masses may be of unequal size and irregularly spaced. In the wider medullary rays secondary vascular tissue is usually formed making the ring continuous. On the other hand, two neighbouring primary bundles sometimes develop so close together that they coalesce, in which case only nine masses are seen. In this case the flower may exhibit some degree of tetramery for the ten vascular masses ordinarily found provide the midribs of sepals, petals and carpels—the stamens may, for the moment, be left out of account. Outside this vascular ring, scattered in the cortex, are numerous small anastomosing strands derived from the border of the ring and forming a peripheral system. This cortical vascular system is continued upwards after the central vascular ring has been used up to supply the cords from which arise successively the midribs of the members of the different whorls, and herein we have proof that in

this species the axis does undoubtedly share in the formation of the cup-shaped structure which carries sepals and petals on its rim¹.

At a slightly higher level the continuous vascular ring breaks up, the primary bundles becoming separate (Fig. 2). Small portions of vascular tissue become detached from the sides of these bundles, the appearance being sometimes such as to suggest that a strand is cut off *on each side of those masses lying on the radius of the petals* (Fig. 3). But there is some indication that the mode of origin of these detached portions is to some extent dependent upon the spacing and is therefore not of fundamental importance in the present connection. Where, as in *Pyrus communis*, small secondary bundles are formed between, and serve to connect some of the primary bundles (see Fig. 1), these secondary bundles may contribute to the detached portion. On the other hand, in the case of *Cydonia japonica* Pers. described below, where the ultimate arrangement is similar, secondary connecting bundles are not usually found and here, as a rule, only a single portion is detached from each primary mass. The two strands referred to above as appearing in *Pyrus communis*, one on each side of the vascular mass on the radius of a petal, at once converge towards the centre, thus giving rise to an inner ring of five pairs of strands surrounding the pith (Fig. 4). This immediate convergence leaves the original 10-radius ground plan still evident. The main cords from which the strands are detached eventually furnish the midribs for the petals while the five detached pairs of strands consolidate later to form five inner semi-solid fertile carpels. As soon as, or even before, the fresh disposition just described is completed a single portion of vascular tissue becomes detached from the side or inner face of the five alternate and now more peripherally placed cords on the radius of the sepals (Figs. 4, 5). These detached portions constitute the midribs of five outer sterile carpels (the only ones recognised hitherto). The main cords from which they branch off are destined to become the midribs of the sepals. We have then at this level an outer circle of ten separate cords which eventually furnish midribs for the members of the perianth; within this ring five carpel midribs consisting of single bundles in line with the sepals; and still nearer to the centre five pairs of bundles opposite the petals. These pairs, as they continue upwards, break up into several smaller strands forming five linear groups delimiting the central pentagonal area of purely

¹ It must not be inferred from this statement that the converse relation necessarily holds good, viz that where no cortical vascular system occurs no axial tissue is present. Further investigation is needed on this point.



Figs. 1-6. *Pyrus communis* L. Flower stalk and receptacle in transverse section at successively higher levels. Figs. 1-4 from one specimen (B), Figs. 5, 6 from another specimen (A).

parenchymatous pith (Figs. 6, 7), the xylem components of each group which border on the pith being gradually reduced to the central couple.

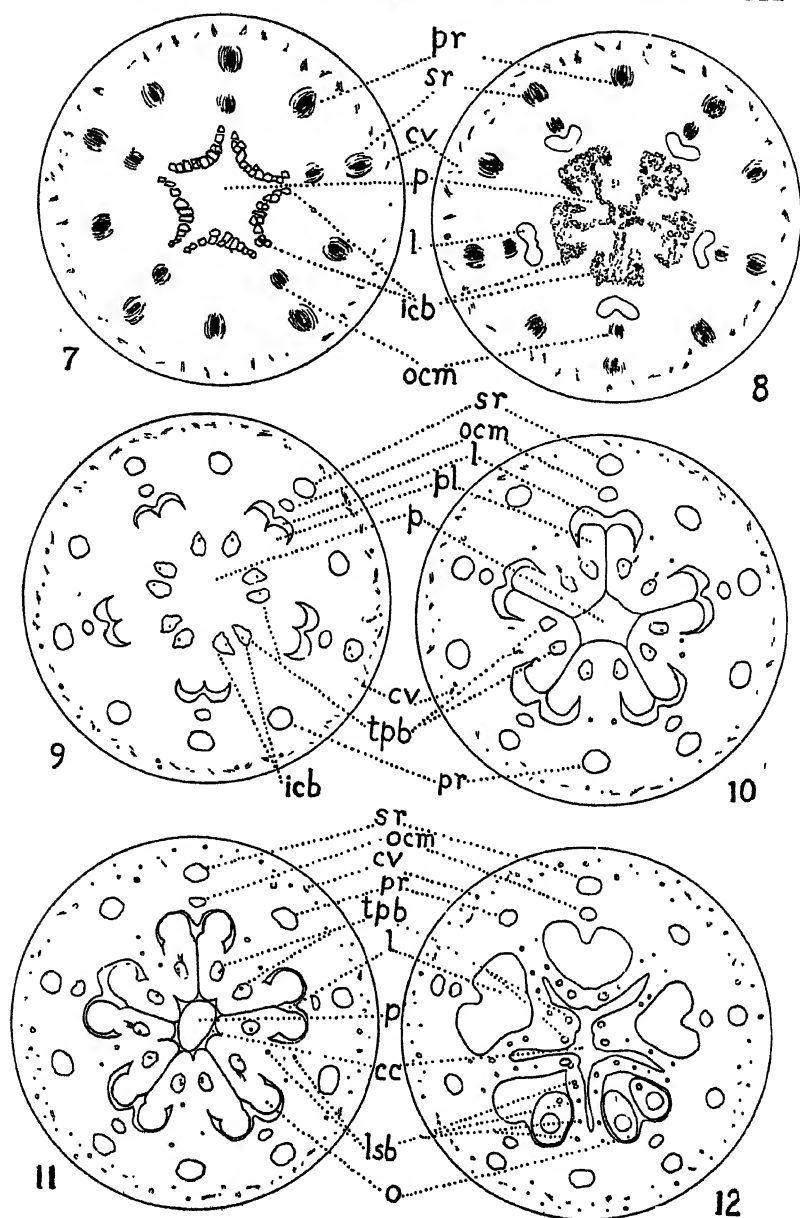
Slightly above this level the now almost purely phloem-containing wing portions of these extended bundle groups wander into the pith forming an irregular 5-rayed star (Fig. 8), and eventually end there blindly. As the twin residual xylem strands have now somewhat diverged while the adjacent phloem wings of neighbouring groups have come into contact in their passage to the centre the primary rays opposite the sepals (Figs. 6, 7) become obliterated, a new broad ray now occurring opposite the petals between each such pair of xylem strands (Fig. 8). About this same level the loculi make their appearance on the inner side of the midribs of the sterile carpels which, as already stated, lie on the radius of the sepals.

As the ovule-bearing region is reached the superfluous vascular tissue is found to have disappeared, the pith once more becomes wholly parenchymatous, and the pair of bundles lying on the radius of the petals and forming the residuum of each original bundle group again stand out distinctly (Fig. 9). They constitute the placental cords, each pair representing the double vascular cord of one of the five fertile carpels forming the inner whorl. Twin ovules are seen in each loculus, one arising from the fertile carpel bounding the one side, the other from the placental bundle belonging to the neighbouring carpel on the other side. *Thus, as has elsewhere¹ been shown to be the case in other families, the two placentae supplying one loculus represent the meeting edges not of one and the same carpel but of two contiguous carpels.*

Immediately above this level the fertile carpels become defined centrally as five arrow-headed structures distinct from each other and delimiting, though remaining continuous with the pith, now again seen as a pentagonal area (Fig. 10). The sterile carpels also become more clearly indicated, each projecting into the loculus as a median protuberance.

Somewhat higher still the pith, representing the apex of the floral axis, diminishes in area, becomes free from the surrounding inner whorl of carpels appearing as an island of tissue (Fig. 11), and finally disappears leaving a central space. At the same time the arrow-head ends of the fertile carpels are seen to be no longer fused along the line of contact, the demarcation line becoming now an actual passage leading from the loculus to the pith cavity. Furthermore, each

¹ See "Carpel Polymorphism, I," *Annals of Botany*, 39, p 166, Jan 1925



Figs. 7-12. *Pyrus communis* L. (continued). All from specimen A.

arrow-head begins to split in two radially, the cleavage starting from the now free inner (central) border and extending outwards along the mid-line of the septum which now shows a few small vascular strands which have been given off from the placental cords (Fig. 11).

As still higher levels are reached the number of these vascular strands increase, those from each placental cord forming a linear series extending the length of the septum (fertile carpel) which thus shows a double row of these bundles, one on either side of the cleavage line which also gradually extends to the septum base (Fig. 12). The free ends resulting from this cleavage, that is to say, the separating halves of one fertile carpel, as they lengthen, diverge from each other, and owing to the expansion of the loculus are now in contact with the half-carpel on either side only by their tips. The passage connecting loculus with pith cavity and separating these tips disappears and the two contiguous pieces fuse (Fig. 12). The new structure thus formed from portions of two carpels is seen as a projection into the pith cavity and contains within itself the remnants of the two respective placental cords together with some of the nearest bundles previously detached from these cords and arranged in linear series.

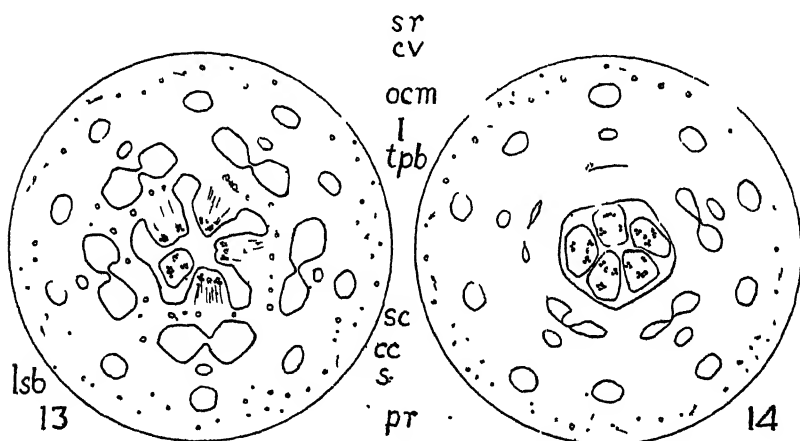
The five projecting structures so formed very soon separate off from the main portion of their respective fused carpel halves (Fig. 13) as they turn upwards to become the stylar filaments which here come into being at a level at which the loculi are still in existence (Fig. 14). In having separate more or less gynobasic styles *Pyrus communis* differs from *Cydonia japonica* and *Pyrus Malus*, two species to which we shall have occasion to refer later in which the style filaments are terminal and conjoined at the base, and have a different construction. For it is to be noted that in *Pyrus communis* the sterile carpel midrib forms no part of the style but continues its upward course on the outer side of the loculus to a point considerably above the level of origin of the style filaments (see Figs. 13, 14). Then, as the loculus comes to an end, it turns inwards and merges with the residual bundles of the linear series which are not utilised in style formation, and, it may be, curves downward behind the loculus.

The free style filaments of *Pyrus communis* continue to show throughout their length one single or two separate dorsally placed vascular bundles arising from complete or partial fusion of the several bundles of the two contributory linear series, and towards the ventral (inner) face two bundles derived from the placental cords of the two component half-carpels, one on either side of the stylar canal.

The stigma, small, discoid, with a central depression and a notch on one side is too simple in form to render its dual origin obvious to the eye, but in *Cydonia japonica* which, though similar to *Pyrus communis* in general ground plan, differs from the latter species in certain important particulars, the elaborate design of the stigmas at once proclaims the composite nature of the style filaments.

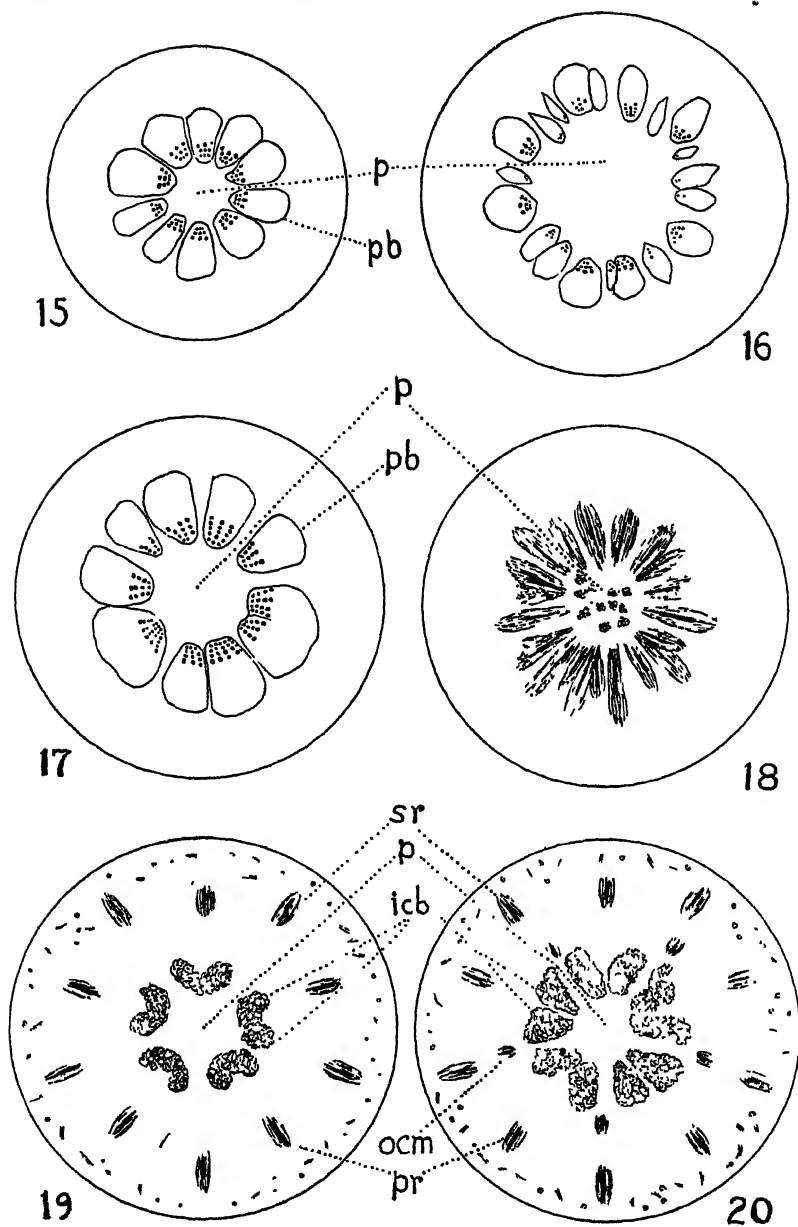
Cydonia japonica Pers. (Figs. 15-33)

In the upper region of the flower stalk as in *Pyrus communis* ten primary vascular bundles are commonly distinguishable but in *Cydonia*, as a rule, there are no interconnecting secondary bundles (Fig. 15). In some flowers the transverse section showed only nine



Figs. 13, 14 *Pyrus communis* L (continued) Both from specimen A

primary masses, two of the ten bundles having developed in such close contact as to have become fused so that the resulting apparently single mass represented a double bundle (Fig. 17). As stated above, only a single portion of vascular tissue seems to be detached from each of the primary bundles as the flower level is reached (Figs. 16, 18). At this stage the construction shows a 20-radius ground plan in contrast with the 10-radius scheme of the flower stalk. The level at which the pith becomes invaded by vascular elements varies. Penetration occurs in some cases as soon as the 20-radius ground plan comes into being (Fig. 18); in others, not until much later when the placental cords have not only taken up a central position but have re-arranged themselves, after divergence of the original bundle pairs

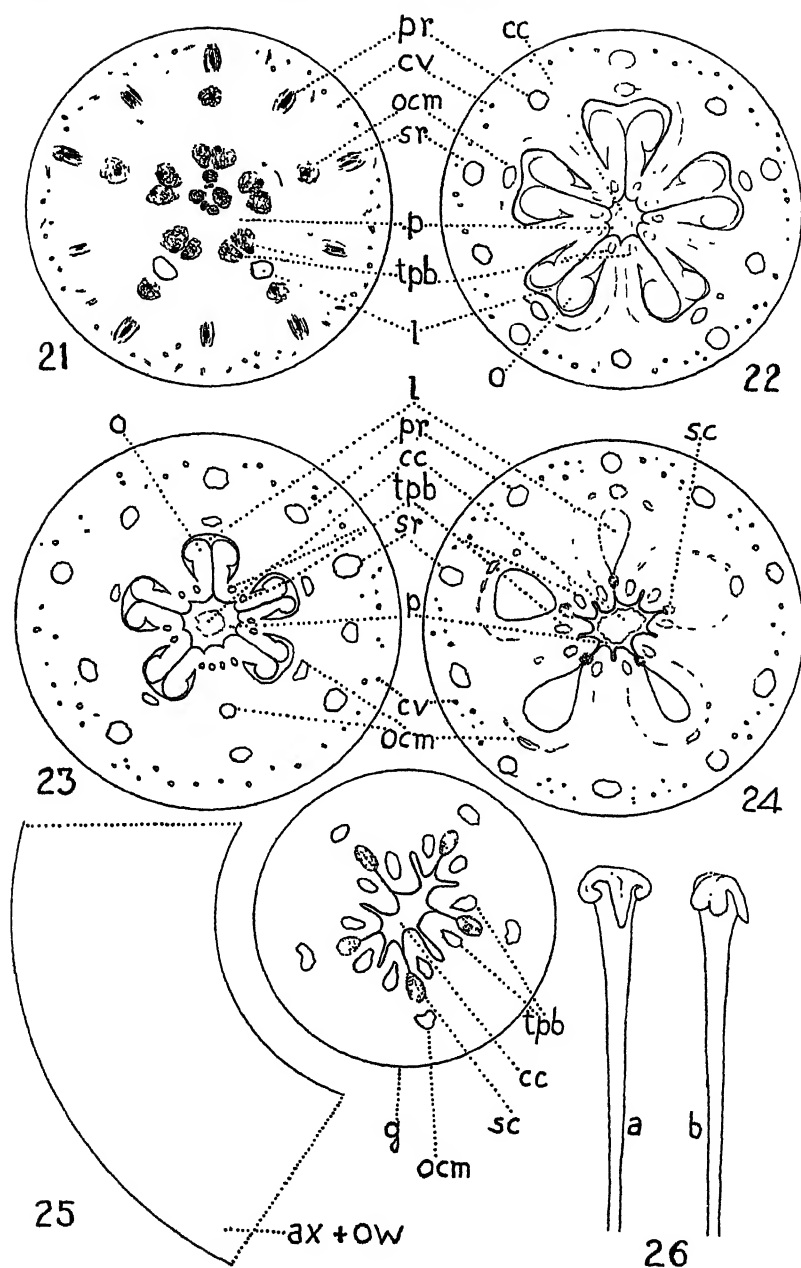


Figs. 15-20. *Cydonia japonica* Pers. Flower stalk and receptacle in transverse section at successively higher levels. Figs. 15, 16 from one specimen (E), Figs. 17, 18, 20 from another specimen (B), Fig. 19 from a third specimen (C).

opposite the petals, in a secondary pairwise grouping *opposite the sepals*, and when the midribs of the sterile carpels have also made their appearance on the same radius. This change of orientation in the portions of vascular tissue detached from the cords on the radius of the petals is particularly clearly seen in *Cydonia* and is shown in Figs. 19-21. In Fig. 19 the detached portions appear as single masses with two xylem strands facing away from each other on their flanks. In Fig. 20 each of these portions has separated into two halves; on the alternate radii the sterile carpel midribs derived from the sepal cords have now made their appearance. In Fig. 21 the superfluous vascular tissue has invaded the pith and the re-pairing of the half-carpels (placental cords) has taken place so that two xylem strands, now closely approximated, face one another on the radius of the sepals; on the same radii are to be seen some of the loculi which make their appearance at about this level.

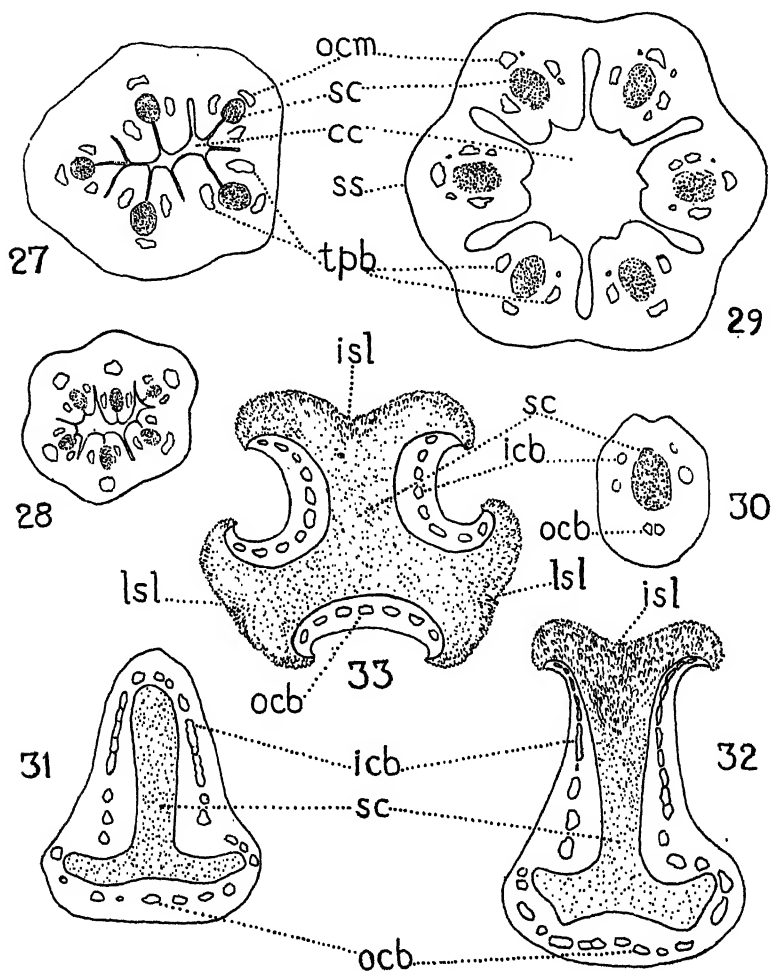
The delimitation of the placental carpels from the pith is not nearly so sharp as in *Pyrus communis*. Whereas, in *Pyrus*, the central parenchyma (floral axis) separates cleanly from the carpels almost immediately after the formation of the loculus (see Fig. 10), and as it diminishes in area until it disappears leaves in its place a space bounded by the five well-defined arrow-headed central ends of the septa (see Fig. 11), in *Cydonia* the axial parenchyma itself tears but remains continuous along its border with the septa, forming a ragged boundary to the central cavity (Fig. 22). Only higher up in the ovule-bearing region does the epidermal tissue of the placental carpels come to form the lining layer of the central space. Another feature characteristic of *Cydonia* is the abundant formation of vascular strands running from the main cords of the fertile to the sterile carpels. These strands are seen cut longitudinally as they run horizontally in the septum to curve round the outer border of the loculus (Figs. 22, 24), whereas, in *Pyrus*, the branches given off from the placentae take an upward and separate course.

The two species also differ markedly as regards the position, origin and behaviour of the style filaments. As the septa in *Cydonia* shorten the loculi gradually disappear giving place to the stylar canals (Fig. 24). The ovary becomes free from the enveloping tissue consisting of the basal portions of the other whorls fused with the axis. With the separation of this outer ring we also part company with the perianth vascular cords whose further course need not concern us (Fig. 25). The sterile carpel midribs now move close up to the canals (Figs. 27, 28); this brings them into the neighbourhood



Figs. 21-26. *Cydonia japonica* Pers. (continued). Fig. 21 from specimen B, Figs. 22, 24, 25 from specimen E, Fig. 23 from specimen A.

of the placental carpel bundles and both vascular systems pass up entire into the style shaft (Fig. 29), a hollow column formed by the joined bases of the style filaments. Each filament shows throughout



Figs. 27-33. *Cydonia japonica* Pers. (continued). Fig. 27 from specimen E, Figs. 28-30 from specimen A, Figs. 31-33 from specimen F (highly magnified).

its length a stylar canal and three bundle groups, one group lying between the canal and the outer convex surface, the other two being distributed pairwise on the two sides (Fig. 30). The style filaments are thus terminal and not, as in *Pyrus communis*, to some extent

gynobasic. Each filament though apparently a simple style is in reality, as in *Pyrus*, a composite structure. Here, however, the construction is more complex than in the Pear for the whole sterile carpel goes to its formation in addition to half the semi-solid carpels on the right and left which alone are present in the case of the latter type. This $\frac{1}{2} \times 1 \frac{1}{2}$ carpel combination is plainly reflected in the curiously formed stigma which shows a long median V-shaped lobe descending the inner face of the apex of the filament, with a bifurcate lobe on each side as well, the bifurcations being shortly coiled (Fig. 26). In a transverse section of the filament taken just below the stigmatic region (Fig. 31) the stylar canal is seen to have assumed a T-shape, the two sets of bundles derived from the two placental half-carpels are strung out on either side of the long arm of the T, those from the sterile carpel being lined out parallel with the short arm. In the stigmatic region itself the stylar canal opens on to the exterior first on the central (Fig. 32) and later on both lateral faces (Fig. 33), stigmatic papillae covering the surface in these regions and curling over the edge of the style apex. This elaborate construction is irreconcilable with the orthodox view that only five carpels are present each of which produces a simple style supposedly the prolongation of a valve carpel midrib, but is quite in accord with a 10-carpelled ground plan combined with polymorphism, and splitting, divergence and re-pairing of the half-carpels of the inner fertile whorl, phenomena which have already been shown to occur in other families (e.g. Droseraceae, Eriocaulaceae¹). This behaviour would seem to be of fairly common occurrence in the non-valve class of carpel, though incompatible, as pointed out in an earlier account of Carpel Polymorphism, with the valve carpel character with which it has hitherto frequently been associated in order to account for appearances which have now been shown to be capable of a different explanation.

Pyrus Malus L.

Only a brief statement concerning this species need be added to the preceding account for in general construction the Apple, though in some respects a reduced type², resembles *Cydonia japonica*. Such differences as are to be found do not affect the conclusions arrived at above. In the specimen examined the flower stalk showed as is frequently seen in the two previously described species only nine

¹ *Loc. cit.* p. 151.

² It differs both from *Cydonia* and the Pear in having only one ovule developed in each loculus.

vascular masses, one of which was obviously formed of two bundles occurring in such close contact as to have become fused. The pith was found to be invaded by vascular elements at a level at which the primary vascular bundles still form the usual ring and considerably below that at which the loculi come into being. Not only does this invasion take place much earlier than in either of the other species described above but the amount of vascular tissue is so great as almost to cause the disappearance of the original parenchyma. Early separation of the central ends of the fertile carpels causes the passage connecting the loculi with the pith cavity to become complete almost as soon as the ovule-bearing level is reached. Owing to these two causes—the inroad of a large quantity of vascular tissue, rapidly followed by the appearance of a central cavity—the stages in which the pith becomes first defined as a pentagonal area, and then as it disappears leaves the free ends of the fertile carpels exposed as arrow-headed structures, are not seen. Instead we pass directly to the stage of a 5-rayed central cavity, as already mentioned, and then, as the fertile carpels themselves begin to split, to the 10-rayed form seen in the two preceding species. Branching of the placental cords leads to the formation of a double linear series of bundles in the septum as in *Pyrus communis*. As the loculi close the sterile carpel midribs bend in and merging with the bundles of this series pass up with them into the terminal style shaft as in *Cydonia japonica*.

The presence of two carpel whorls thus seems to be clearly established for the three species under consideration which are probably illustrative of many other Rosaceae. This, together with the polymorphic character of the whorls, enables us to explain an otherwise puzzling anomaly which is met with now and again in these types, viz. the occurrence of a larger number of style filaments than there are loculi. This peculiarity has been noted by Church¹ in the case of *Cydonia japonica*, and his statement that seven and eight style branches may occur when the five carpels remain constant affords a good illustration of the kind of paradox with which one is inevitably and constantly confronted on the orthodox view which recognises only the valve carpel. In this particular case the presence of these additional style branches (so-called) furnishes the most convincing proof that the septum with its divergent twin placentae constitutes a separate carpel. For in an ovary which bears e.g. six style filaments but has only five loculi it will be found that one of the septa is in reality double, being broader than the other four, and that it shows

¹ *Types of Floral Mechanism*, p. 170.

at its outer end a strong vascular cord not seen in the others and at its inner extremity two pairs of placental bundles instead of one (Fig. 23). The double septum is in fact made up of *two* semi-solid carpels between which the alternate solid sterile carpel represented by the peripheral vascular cord has been unable to develop the loculus, owing presumably to restriction of space. In the case of genuine valve carpels we should probably be safe in taking the number of loculi as a guide to the carpel number, but this is not a reliable index when the carpels are of the solid class. In a six-styled flower of the type described the gynœcium, its five loculi notwithstanding, is obviously hexamerous in both whorls. This is shown in Figs. 28 and 29 which are taken from the same flower as Fig. 23.

In the case of *Cydonia* and the Apple and Pear we are dealing with types which are isomerous throughout and in such cases the midribs of the outer carpel whorl are always derived from the sepal cords and, like the loculi, always stand in line with them. But in some allied forms, as e.g. *Pyrus Aucuparia* Gaertn. (Mountain Ash), the number of carpels in each whorl is reduced to four or sometimes to three. This want of conformity between the carpellary and the perianth whorls would necessarily result in pronounced asymmetry if the same regular mode of origin of the carpels was followed as in the strictly isomerous types. It is therefore interesting to find that the relation between the outer carpels and the perianth members is quite plastic, and that one or possibly two of the four outer carpel midribs are in these circumstances derived from a petal cord. In this way the inevitable asymmetry is in part compensated, and as equal distribution in space as may be is obtained of carpels, and hence also of loculi, despite the non-conformity of the numbers in the whorls.

Although the arrangement of the staminal whorls is not of primary concern here brief reference to their disposition may be made in passing. The outer whorls at least appear to be 10-membered. This is accounted for by the fact that each petal cord gives off a bundle right and left. These ten bundles form the cords of the outer staminal whorl which alternates with the perianth. A third segment detached from the inner face of the petal cords and a corresponding single cord from the inner face of the sepal cords furnish the second whorl which alternates with the first. A repetition of this scheme, in whole or in part, provides for the very variable total number of stamens (20 to 60 or more) found in the different species of *Pyrus* and *Cydonia*.

SUMMARY OF CONCLUSIONS

1. The perigynous and syngynous (epigynous) flower types are fundamentally distinct, for whereas there is clear proof that in perigyny the floral axis becomes concave and takes part in the formation of the ovary wall, the evidence available is against the accepted view that these relations hold in the case of syngyny (epigyny).

2. In the Rosaceous types investigated (*Pyrus* and *Cydonia*) and probably in others, the carpels are in two whorls of five and are polymorphic. Those of the outer whorl are solid, sterile and in line with the sepals and loculi; those of the inner whorl are fertile and semi-solid, and lie on the radius of the petals.

3. The concave form of the floral axis probably accounts for the fact that the vascular cords for the carpels are given off before those for the perianth and androecium become separate, and that those for the inner carpel whorl are detached before those for the outer whorl.

4. In isomerous types (*Pyrus communis*, *P. Malus*, *Cydonia japonica*) the outer carpel midribs are given off from the vascular cords destined for the sepals, hence whatever the number of staminal whorls these carpels and the loculi always stand opposite the sepals. In forms in which the number of members in the carpel whorls is fewer than in the perianth whorls (*Pyrus Aucuparia*) one or two of the outer carpel midribs are derived from the petal cords and the others from the sepal cords. In this way the asymmetry is reduced.

5. The style filaments do not represent simple styles. When more or less gynobasic (*Pyrus communis*) they consist of the adjacent halves of two split carpels (semi-solid) of the inner whorl; when terminal (*Pyrus Malus*, *Cydonia japonica*), of one whole outer (solid) carpel conjoined with the contiguous half of the inner (semi-solid) carpel on each side.

6. This composite construction is reflected in the complex stigma of *Cydonia japonica* which is in the form of a median V-shaped lobe with a bifurcate coiled lateral lobe on each side.

7. In isomerous pentamerous types flowers are occasionally found with six style filaments although only five loculi are formed. In such cases one septum is double with two pairs of placental cords instead of one at the inner end, and with an additional vascular cord at the outer end representing the sixth member of the outer whorl which has no corresponding loculus. The sixth style filament is thus accounted for and its presence furnishes further proof that there are two carpel whorls and that the carpels are polymorphic.

I desire, in conclusion, to tender my very grateful thanks to Miss D. F. M. Pertz who kindly made the drawings here reproduced.

The expenses incurred in connection with the present work and with the previous work on the inferior ovary have been defrayed by a grant from the Royal Society.

EXPLANATION OF FIGURES

Fig. 1. The central ring of vascular tissue is continuous consisting of ten primary bundle masses connected here and there by secondary bundles; outside this ring are the small scattered bundles of the cortical system.

Fig. 2. The central ring is breaking up, small portions of vascular tissue are becoming detached from the primary bundles.

Fig. 3. Most of the primary bundle masses are now separate and some of the small intervening portions are also completely detached; the five primary bundles on the radius of the sepals project slightly further outwards than the alternate five on the radius of the petals.

Fig. 4. The ten primary bundles are cut obliquely as they bend outwards as the flower stalk forms the receptacle. The intervening detached portions have moved towards the pith and have taken up their position in pairs on the inner face of the main bundles on the radius of the petals, the xylem strands being next the pith. Single portions are also becoming detached from the alternate primary bundles on the radius of the sepals.

Fig. 5. The latter process has been completed. A single detached bundle now lies to the inside of each of the five outer bundles (which furnish the midribs of the sepals) and a pair of bundles to the inside of each of the alternate bundles (which furnish the midribs of the petals). All the bundles have resumed an upward course and are cut transversely. [The xylem is not shown except in the bundles of the inner ring.]

Fig. 6. The ten bundles which furnish the perianth midribs (outer ring) and the five bundles (=midribs of the outer sterile carpels) lying on the same radius as, and to the inside of the sepals turn outwards and are cut obliquely; the five pairs of bundles forming an inner ring (=twin cords of the inner fertile carpels) have broken up into smaller bundles.

sr, radius of sepal; *pr*, radius of petal; *p*, pith; *pb*, primary vascular bundles; *ca*, cortical vascular system; *ocm*, midrib of an outer sterile carpel; *icb*, bundles of an inner placental carpel.

Fig. 7. As Fig. 6, but the breaking up of the inner ring of five pairs of bundles has been carried further, the group derived from each pair now forms an arc bounding the pith on the radius of each petal leaving a broad medullary ray on the radius of each sepal.

Fig. 8. The loculi have made their appearance on the inner face of the five bundles lying to the inside of the sepal midribs. [These bundles furnish the midribs of the outer sterile carpels.] The original five bundle pairs on the radius of the petals after breaking up (Figs. 6, 7), have re-paired; the new pairs, with two xylem strands facing away from each other, consist of one component from the neighbouring original pair opposite a petal on the one side, and a similar component from the corresponding adjacent pair on the other side; consequently the main medullary rays now lie opposite the petals. The portions of the original bundle pairs (mainly phloem) not required for the carpels are invading the pith.

Fig. 9. The discarded vascular elements which end blindly in the pith have disappeared. The placontae are becoming differentiated, one of each pair being supplied by one of the original twin bundles (half-carpel) on the one side, its

fellow from a similar bundle (half-carpel) on the other side. The sterile solid carpels of the outer whorl project into the loculi as a rounded protuberance.

Fig. 10. The inner fertile carpels have become delimited along their contiguous flanks and at the centre (where they are still however continuous with the pith) and appear as arrow-headed structures; small vascular strands given off from the placental cords are seen in the septa.

Fig. 11. The fertile carpels have separated completely from the pith (= apex of floral axis) and along their flanks so that a passage now connects the loculus with a central space; they have also begun to split in the radial direction from the centre outwards.

Fig. 12. The fertile carpels have split almost to the base of the septum; each resulting half-carpel fuses at its central end with the adjacent half of the neighbouring carpel, thus cutting off the loculus from the central cavity from which the pith has now disappeared. The linear series of bundles detached from each placental cord extends the whole length of the septum.

tbb, twin placental cords; *lsb*, linear series of bundles derived from a placental cord; *l*, loculus; *o*, ovule; *pl*, placenta; *cc*, central cavity; other lettering as in the previous figures

Fig. 13. The half-carpels derived one from each of two neighbouring fertile carpels have now fused for about half the length of the septum. In one case the projecting end of the fused region has become free forming a style filament. The fused region contains the two placental cords (between which the stylar canal is appearing) and some of the nearest of the other bundles (seen cut longitudinally) of the septum.

Fig. 14. All five stylar filaments are now free. The loculi, though becoming smaller, have not yet disappeared.

s, style; *sc*, stylar canal; other lettering as in the preceding figures.

Fig. 15. The fibro-vascular ring is composed of ten separate primary bundles without intervening secondary bundles.

Fig. 16. Portions of vascular tissue have been detached from the primary bundles so that there are now twenty bundles.

Fig. 17. Similar to Fig. 15, but the fibro vascular ring shows only nine primary bundle masses, one being obviously double.

Fig. 18. Similar to the stage shown in Fig. 16, but the bundles are bending outwards and are therefore seen cut obliquely. Only nineteen bundles are distinguishable. [When the two bundles derived from the original double primary bundle mass (shown in the same position as in Fig. 17) have drawn apart completely another portion of vascular tissue will be detached between them, bringing the total number of bundles up to twenty.] A few vascular elements have wandered into the pith.

Fig. 19. The ten vascular bundles which furnish the midribs of the sepals and petals form an outer circle. On the radius of those destined for the petals (- the five lying further from the surface than the alternate five) is an inner ring of five bundle masses belonging to the five inner fertile carpels. On the alternate radii opposite the cords destined for the sepals (- the five lying nearest to the surface) are five broad medullary rays. The cortical vascular system has now made its appearance.

Fig. 20. The bundle masses of the five fertile carpels have split in two, the xylem strand in each half facing away from that in the other half. Hence, when the two halves of one fertile carpel later diverge, the xylem of the one half faces towards that of the adjacent half of the neighbouring carpel (see Fig. 21).

The lettering as in the preceding figures.

Fig. 21. The twin bundles belonging to each fertile carpel and originally forming a pair on the radius of each petal (compare Fig. 19) have diverged and

re-paired on the radius of the sepals, each new pair consisting of one half of a carpel and the adjacent half of a neighbouring carpel. The new pair supply the two placenta of one loculus. The portions of vascular tissue not required for the placental cords invade the pith. Two of the loculi have already made their appearance and the position of another is indicated.

Fig. 22. The fertile carpels have become delimited on their inner face and flanks as arrow-headed structures. The pith, now again free from vascular tissue, does not separate cleanly from the fertile carpels as in *Pyrus communis* (compare Fig. 11) but becomes torn in the centre, the remnant remaining attached to the inner face of the arrow-heads as a ragged fringe which thus cuts off this central cavity from the channel leading from the loculus. Detached bundles from the placental cords are seen extending down the septum and around the loculus.

Fig. 23. The same stage from a specimen with five loculi and six style filaments. One septum is seen to be double with two pairs of placental cords at its inner end and an additional single strong vascular cord (=sixth outer carpel) at its outer end; the ovary in this specimen is thus hexamerous in both whorls.

Fig. 24. The loculi are closing up and the stylar canals have appeared between them and the median split in each fertile carpel.

Fig. 25. The gynoecium has become free from the surrounding tissue of the other whorls and the conjoined axis, only a sector of which is shown and that in outline alone. The pith has now wholly disappeared leaving a central ten-rayed cavity.

Fig. 26. Two views of the upper free end of a style filament (*a*) from the inner face showing the central V-shaped stigmatic lobe, (*b*) from the side showing one of the bifurcate lateral lobes.

g, gynoecium; *ax + ow*, axis + other whorls; other lettering as in the preceding figures.

Fig. 27. Apex of ovary. The outer carpel midribs have now moved inwards and lie close to the stylar canals.

Fig. 28. The same stage from the specimen shown in Fig. 23. Both carpel whorls are hexamerous although only five loculi are present (see Fig. 23).

Fig. 29. Style shaft from the same specimen as Fig. 28 composed of the conjoined basal portions of six similar style filaments. Each filament base shows a central stylar canal, and three groups of vascular bundles, one behind, and one on each side of the canal.

Fig. 30. One of the style filaments after it has become free.

Fig. 31. Through a style filament just below the stigmatic region. The style filament and the stylar canal appear T-shaped, the short arm of the T is towards the outer side, the long arm points to the flower centre.

Fig. 32. Through the lower stigmatic region. The long arm of the stylar canal has opened on to the inner surface where the lining cells form the stigmatic papillae of the V-shaped stigma lobe.

Fig. 33. Through the upper stigmatic region. Both short arms of the stylar canal have also opened on to the surface, the lining cells of the canal being now continuous with the two lateral stigmatic lobes as well as with the median inner lobe. Alternating with the three stigmatic surfaces are the non-stigmatic areas in which are distributed the three groups of vascular bundles.

ocb, outer carpel bundles; *isl*, inner stigmatic lobe; *lsl*, lateral stigmatic lobe; other lettering as in the preceding figures

ON THE GEOGRAPHICAL DISTRIBUTION OF THE STYLIDIACEAE

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(With 3 figures in the text)

THE Australasian family of the Stylidiaceae is a very natural and homogeneous group of flowering plants sharply separated by certain structural features from all other families of Angiosperms. The most important of these features lies in the organisation of the genitalia. The androecium consists of two stamens only and these are completely fused with the style to form a column or gynostemium having the anthers sessile one on each side just below the stigma. In the vast majority of species this column is irritable and there is a very complicated and peculiar pollination mechanism. In all but one monotypic genus the corolla is markedly zygomorphic, the anticus lobe forming a small, often cucullate, labellum. The members of the family are small to medium-sized annual or perennial herbs, rarely suffruticose, with small, entire, usually imbricate or rosulate leaves. The corolla is generally conspicuous. The seeds are rather small and there appears to be no definite dispersal mechanism.

According to Bentham and Hooker the family contains five genera—*Phyllachne*, *Forstera*, *Oreostylidium*, *Levenhookia* and *Stylidium*. Engler included the genus *Donatia*. This genus has also been considered to belong to the Saxifragaceae and its systematic position is not at all certain. On this account and because it does not show any of the peculiar features mentioned above, it is omitted from further consideration in this paper. This omission has no effect upon the distribution of the family as a whole since the range of *Donatia* is paralleled exactly by that of *Phyllachne*. The group of genera considered here is, thus, the Stylidiaceae of Bentham and Hooker and the Stylidioidae of Engler.

The family has been monographed by Mildbraed in the *Pflanzenreich* series and this work has been the source from which most of the data in this paper have been obtained. Nine species of *Stylidium*, described since the monograph, are included in the statistics.

The relationships and differences between the genera are best shown by means of the following clavis:

A. Anthers curved, monothealous.

(a) Flowers sessile apical, leaves closely adpressed. **Phyllachne** 4 spp.

(b) Flowers pedicellate, leaves basal, adpressed or spreading.

Forstera 4 spp.

B. Anthers ditheous.

(a) Corolla regularly 5-lobed.

Oreostylidium 1 sp.

(b) Corolla irregularly 5-laciniate with a labellum.

* Labellum stipitate, column non-motile

Levenhookia 6 spp.

** Labellum sessile, column irritable.

Stylidium 112 spp.



Fig. 1. Sketch-map showing the distribution of the genera of Stylidiaceae.

1. *Stylidium*. 2. *Levenhookia*. 3. *Forstera*. 4. *Oreostylidium*. 5. *Phyllachne*

DISTRIBUTION OF THE FOUR SMALL GENERA

Phyllachne contains four species, all much branched herbs with muscoid habit, a complete covering of small adpressed leaves and small sessile apical flowers. The specific differences are those of leaf form. *P. uliginosa* is a native of the Magellan region of South America and is the sole representative of the family in the New World. The other three species are found in New Zealand; *P. Colensoi* in North, South and Stewart Islands, the other two only in the South Island.

Forstera also contains four species, erect or decumbent perennials with pedicellate flowers and small imbricate leaves. *F. bellidifolia* occurs in Tasmania. The other three species are all New Zealand endemics, one in South and Stewart Islands and two in both North and South Islands.

Levenhookia has six species, small erect annuals with minute scattered leaves and simple or corymbose racemose inflorescences. This genus (in affinity close to *Stylidium*) is exclusively Australian. Five species are found in the south-west corner of the continent only, while the sixth extends thence to South Australia and Victoria.

Oreostylidium is a monotypic and possibly primitive genus, lacking the zygomorphy of the others. *O. subulatum* is a dwarf acauline perennial herb with sessile flowers and subulate leaves and is confined to the South Island of New Zealand.

DISTRIBUTION OF THE GENUS *STYLIDIUM*

Stylidium is by far the largest genus in the family and contains no less than 112 species. Geographically it is almost entirely Australian, all but four of its species being confined to that continent. Of these four, two are Asiatic and entirely extra-Australian, *S. tenellum* being found in Burma, Malacca and Tonkin, and *S. Kunthii* in Bengal and Burma. A third species, *S. uliginosum*, occurs in Queensland and also in Ceylon and on the south coast of China. The fourth species, *S. alsinoides*, is found in north-east Australia and Queensland and also in the Philippines (*vide* specimens in Herb. Mus. Brit. and Herb. Kew).

There thus remain 108 species distributed over the continent of Australia. When the distributions of these are plotted it is at once seen that they are by no means evenly scattered over the whole region. The centre is quite devoid of species, as is also the north-west coast. All the species are in fact concentrated in a rather broad coastal zone reaching from the north coast along the north-east, east and southern coasts to the south-west corner of the continent. This belt is, however, not uniform in width, but is almost broken in the middle part of the south coast and its continuity here is only maintained by one or two species which range from south-west Australia to New South Wales.

Within this coastal belt the species themselves are not at all evenly scattered, but are grouped in the following way. In the north-west is a group of some eleven local species in the basin of the Victoria River. A second but wider group extends from the north-west across to the east coast of the Cape York Peninsula. A third group of about eight species, with again rather wider areas, occupies the east coast as far south as New South Wales. In Victoria and eastern South Australia are a very few local species. The last group is that of the south-western corner of the continent, a region with no fewer

than 77 species. The total area of this group is scarcely as large as the average individual areas among the species of the east and the different species overlap to an amazing degree.

All these groups are connected by the areas of a few comparatively widely distributed species. Three such join south-west Australia with Victoria and Tasmania: one species, *S. graminifolium*, connects eastern South Australia with Queensland and also with Tasmania. This island has only three native species. A single species, *S. Tepperianum*, is entirely confined to Kangaroo Island near Adelaide.

Broadly, the total continental range of the genus is divided into two well-marked parts, a much larger northern, eastern and south-eastern part populated by comparatively few rather wide-ranging species and a much smaller south-western part populated by an enormous number of very local species.

DISTRIBUTION OF THE SUB-GENERA AND SECTIONS OF *STYLIDIUM*

The genus is first divided into species with stipitate stigmata and species with the stigmata sessile between the anthers.

The first division comprises one sub-genus only, *Centridium*, and contains only the two species *S. calcaratum* and *S. perpusillum*. These are two of the species with wide ranges which unite western and eastern Australia. The latter reaches Tasmania. They are small or dwarf annuals.

The second division, which contains the remaining species of the genus, is divisible into five sub-genera differing chiefly in the structure of the ovary and capsule.

The sub-genus *Forsteropsis* contains two species, *S. Preissii* and *S. imbricatum*, small perennials with imbricate leaves and general habit resembling that of the genus *Forstera*. Both are confined to the south-west of the continent.

The sub-genus *Andersonia* contains 14 species with solitary flowers or lax corymbose inflorescences. Eleven are entirely Australian and occur in the north of the continent only. Two are Asiatic species (*S. tenellum* and *S. Kunthii*) and the last is *S. uliginosum* found in Queensland and also in Ceylon and China.

The sub-genus *Alsinooides* contains three species, *S. alsinoides*, *S. cordifolium* and *S. tenerrimum*. They are delicate annuals with broad thin leaves and the two former are very closely related. All three are species of northern Australia, but *S. alsinoides* is also found in the Philippines.

The remaining two sub-genera contain very many more species and show far greater range of development than do those already mentioned. Differences of habit together with variations in the ovary and capsule structure are used to subdivide them.

The sub-genus *Tolybangium* is the largest of all, containing, as a whole, 65 species. Within the sub-genus the first section (*Despectae*) has seven species, six local south-western and one, *S. despectum*, all along the south coast to Tasmania and New South Wales. The second section (*Debiles*) has four species in northern Australia and Queensland, one also extending further to the south-east. The third section (*Floodia*) has two species, *S. Floodii* of northern Australia and Queensland, and *S. rubriscapum* of the Kimberley district. The fourth section (*Sparsiflorae*) has three species, one in the south-west and two in northern Australia and Queensland. The fifth section (*Repentes*) has only two species, both low creeping perennials of the south-west. The sixth section (*Guttatae*) has only one species, *S. guttatum*, characterised by its subcapitate inflorescence. It is confined to the south-west. The seventh section (*Junceae*) has also only one species, *S. junceum*, of rush-like habit and with a south-western distribution. The eighth group (*Verticillatae*) has two species with leaves in whorls and in one case a scrambling habit. Both are found in the south-west. The ninth section (*Limbatae*) has but a single species, *S. limbatum*, a stout perennial confined to the Coolgardie region. This is one of the desert species. The tenth section (*Saxifragoidae*) has 20 species, all stout perennials with a Saxifrage-like habit. All are confined to the south-west. The eleventh section (*Linearcs*) has 11 species of similar habit. Six are local south-western, one is confined to Kangaroo Island, three are local south-eastern, while the last is a wide eastern species from Queensland to South Australia and Tasmania. The twelfth section (*Squamosae*) has 11 species all of similar habit and all confined to the south-west.

The sub-genus *Nitrangium*, the second largest, has 26 species. The first section (*Appressae*) has only one species, *S. appressum*, a south-western dwarf or low perennial. The second section (*Sonderella*) has five species, all acauline scapose perennials confined to the south-west. The third section (*Thyrsiformae*) has 16 species similar in habit and in range to the last. The fourth group (*Rhyncangium*) has four species, stout perennials or herbaceous undershrubs, all confined to the south-west.

The above classification is that used by Mildbraed. So far as possible, newer species have been inserted in their proper positions.

The foregoing account of the distribution of the Stylidiaceae is of interest, not only geographically but also because it exemplifies many important features of plant distribution. In the following pages some of the more important of these features are elaborated and discussed.

The markedly discontinuous and disproportionate distribution of the Stylidiaceae, with a thickly populated centre in Australia and an isolated outlier in South America, is an example of a not uncommon phenomenon of plant geography. Such cases at once introduce the difficult question of former geographical configuration and the possible existence of land bridges. The postulation of such bridges is attractive because of the ease with which, by its help, certain otherwise puzzling facts can receive an explanation. At the same time, the direct evidence for such a view is usually scanty. It is, of course, unnecessary to suggest complete and direct land connection; it will be sufficient if it can be shown that at some time there existed no barrier to distribution greater than could be surmounted by plants of average dispersal potentialities. Similarly, in dealing with the flowering plants, it must be assumed that such changed conditions persisted at such time as an Angiospermous flora was widely spread. By means of the explanation of former land connection the distribution of such a genus as *Phyllachne* can be explained as being the remnants of an earlier and continuous distribution. In default of this view there are two other possibilities, namely, that the present range is due to abnormal or accidental transport or that the same plant-type has arisen quite independently in two widely separated places. The latter may be dismissed at once as being entirely without evidence or analogy. The question of transport is more important. Such an explanation is always possible and is one which is extremely difficult either to prove or to refute. Individual features must be considered and in the case of *Phyllachne* neither the New Zealand nor the American plants appear to be particularly liable to such transport. The most important point, however, is that this genus by no means stands alone and is only one of many others with a similar distribution. As the number of such increases so the likelihood of accidental transport decreases, until a point is reached at which the accumulated evidence is in favour of the view that the phenomenon is due to former land connection of some kind. It must also be borne in mind that if accidental transport over wide intervening spaces is at all a common occurrence it will be reflected all over the world by a far greater intermingling of geographical types than is known to

be the case. Two other facts go to support the hypothesis of former land connection. Antarctic exploration has shown that the present South Pole is at the centre of a huge and continuous circumpolar continent now almost completely ice-covered. In the longitude of South America the northern shore of this continent is at a latitude of 62 degrees south and is only separated from continental South America by about 650 miles of sea. In the longitude of New Zealand the Antarctic shore is 65 degrees south and separated from the more northerly land masses by more than 1200 miles of sea. The small Macquarie Island lies about midway between the two. The next point is the very great difference at similar latitudes in the two hemispheres. In the southern hemisphere no flowering plants are known to exist south of latitude 60 degrees. Only two species have been found on the Antarctic continent and both these occur only on the north coasts of Graham Land. The Antarctic circle lies at a latitude of 66 degrees south. In the northern hemisphere conditions are very different. Spitzbergen, which lies across the meridian of 80 degrees north, has no less than one hundred species of flowering plants and the Arctic circle lies but a little way north of Scotland. Were the conditions in the two hemispheres reversed it would excite little wonder if a number of plants were known having distributions from South America along the north shore of the Antarctic continent to New Zealand and the islands to the south. Thus, in order to explain the distribution of *Phyllachne* and other genera, it is only necessary to assume a climatic change of some magnitude and a possible narrowing of the barrier to the south of New Zealand. There is little difficulty in the first assumption. During the Swedish Antarctic Expedition two fossil floras were found in Graham Land. One of these is of Jurassic age and contains many ferns and conifers but the other is of Upper Cretaceous or early Tertiary age and shows a general facies closely resembling that seen in many northern fossil floras of similar date, including such genera as *Sequoia*, *Araucaria*, *Drimys*, *Fagus* and *Knightia*. These discoveries show that great climatic changes have occurred in this part of the world and, more important still, that they have occurred since the time when an Angiospermous flora became established. With regard to the second point it is significant that bathymetric maps of the Southern Ocean show traces of a ridge between New Zealand and the Antarctic continent.

One feature of the present distribution of the Stylidiaceae supports the view that their former relationships, whatever their nature, were across the South Pacific and not across the South Atlantic and

Southern Oceans. In passing westward from the South American end of the family range the various genera exhibit an overlapping distribution. *Phyllachne* ranges from South America to New Zealand, *Forstera* from New Zealand to Tasmania, *Stylidium* joins Tasmania to south-western Australia and *Levenhookia* is confined to south-western Australia. If, now, the reverse direction is considered and South America is taken to be the western limit of range a curious result is apparent, namely that the South American *Phyllachne* is nearest, geographically, to the very different genus *Stylidium*, and is nearer every other portion of the family than it is to the remaining species of its own genus.

All the above facts lead to the conclusion that in the Stylidiaceae we have a family whose floral relationships are across the South Pacific and the present range of which is quite possibly the remains of a much larger one at a time when, under more genial conditions, the great Antarctic continent supported a varied Angiospermous flora.

The next important aspect of the distribution of the Stylidiaceae is the very great difference in size between the various portions of the family. One of the two sub-families is fifteen times as rich in species as the other. This is due to the very great development of the genus *Stylidium*, which has seven times as many species as the rest of the family put together. At the same time, the family consists of only one type of plant, the annual or perennial, small to medium-sized herb and the general growth form is very constant. Were it otherwise it might be suggested that certain factors have favoured the production of one type above the others. It may certainly be argued that *Stylidium* itself does differ from all the rest in the possession of a very peculiar and specialised floral mechanism, but it can hardly be thought that this is in itself sufficient to cause a greater tendency towards species production. The systematic description of an unusually large number of species is but one means of expressing what is really a greater inherent tendency to variation within the given plant group. Unfortunately, little or nothing is known as to the conditions and factors which encourage or inhibit such a tendency or even whether such factors exist. It does seem, however, that such a case as that of *Stylidium* may well illustrate a particular biological condition in which the general processes of inheritance are in a more plastic phase than usual. For this no cause can at present be assigned but the conception of such a possible explanation is important. One possibility is that with variation as

with other biological phenomena there is a certain combination of edaphic factors which allows the process to proceed at an optimal rate. Another possibility is that such a great production of species marks a certain phase in the history of the plant type at which an increased or maximum vitality is shown and when there is the greatest tendency towards variation. At the same time the absolute measure of such a maximum is clearly very different in different parts of the same circle of affinity. The genera *Phyllachne* and *Forstera* seem at present to be in a more or less static condition in which they are maintaining their individuality on a small scale and there is no evidence to show that they have ever experienced a development comparable to that of *Stylidium* to-day. At the same time, there are a number of well authenticated cases of genera which to-day are but the remnants of a much greater development in the past. Such examples as these, together with such cases as *Stylidium*, suggest that possibly most floral types pass through a more or less definite cycle commencing with a gradual increasing development up to a maximum which is followed by a gradual decline to which the logical conclusion is extinction. It must be remembered in this connection that such a "life" of a plant type is a purely artificial period and represents only that part of its total phylogeny during which it exhibits that certain combination of characters which accords with its present systematic definition.

Whatever the practical value of the above remarks they certainly indicate three possibilities which must be taken into account in theoretical considerations on the subject of plant distribution. These are, first, that great and rapid species production may be partly due to a particular combination of external factors and hence may have a geographical significance, second, that species numbers are in themselves insufficient to indicate differences of age between related plant types and, third, that species, even within a single genus, may not be produced at a uniform rate over the whole of a distributional area. The distribution of the Stylidiaceae illustrates all these points.

Perhaps the most obvious and striking fact in the distribution of the continental species of *Stylidium* is their restriction to a coastal belt of varying width almost encircling the continent. The explanation appears to lie in the distribution of the rainfall. In general terms the great centre of Australia is dry and in all directions from it the precipitation increases as the coast is approached. The dry centre is, however, not the geographical centre of the continent but lies rather to the west of the middle, the eastern half being wetter than the

western. Round the drier portion the isohyets form a series of roughly concentric circles, the intervals between them being wider on the east. This simple configuration is complicated by certain islands of higher fall where the land is higher. The zone of greatest precipitation is on the eastern coast of the York peninsula (over 80 inches annually) and this is the part of the continent furthest away from the centre of the western desert (about 5 inches annually). Owing to the indentation of the north-west coast of the continent and the presence of the Great Bight on the southern coast the higher isohyets are discontinuous and broken in two places. Hence there are two distinct

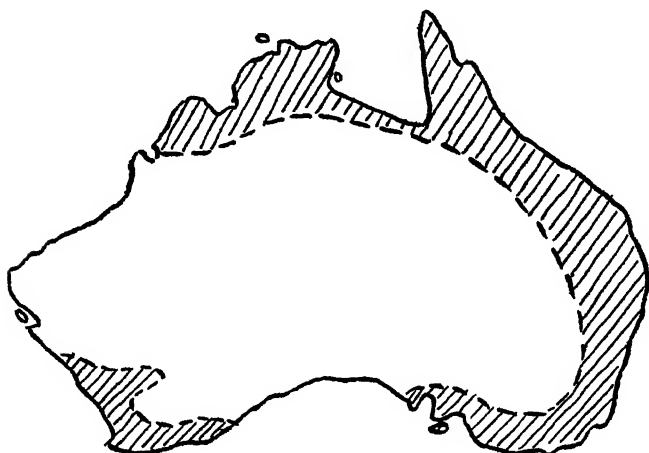


Fig. 2 Sketch-map of Australia, showing (shaded) the distribution of the genus *Stylidium* within the continent.

regions in which the rainfall exceeds 20 inches—a wide belt stretching round the north, east and south-east coasts and a much smaller area in the south-west corner.

These two areas are exactly the two areas inhabited by species of *Stylidium*. If the known localities throughout the genus are plotted on a map it is at once seen that practically all fall within this region and the great majority within the region of even higher precipitation (over 30 inches annually). Again, all localities with the highest *Stylidium* populations are within the latter. Two slight exceptions serve only to emphasize the general correlation: in the Kimberley district of the north-west coast one or two species are found farther

inland on the edge of drier zones, while in the south-west four species, three of which are closely related, are found in the Coolgardie district on the south-western edge of the great desert. These four species belong to one of the more specialised sub-divisions of the genus.

From this there seems little doubt that the limiting factor in the distribution of the genus *Stylidium* within the Australian continent is one of rainfall. The actual figure of limitation probably varies for different divisions of the genus and it also appears to be somewhat lower in the case of the south-western species than it is in the eastern species, although the bulk of the south-western species are found in



Fig 3 Sketch-map of Australia, showing distribution of annual rainfall (approx)

the wetter extreme south-western corner. There is also a difference in the seasonal distribution of the rain. In the east there is a summer and autumn rainy season approaching the tropical type; in the south-west there is winter rain and a dry summer. The slight differences of habit and form between the species of the two regions are probably correlated with this feature. There is apparently no upper limiting figure in the rainfall. *Stylidium* is found wherever the fall is above a certain amount and the extra-Australian species are found where the fall is even higher than it is on that continent.

The xerophytic tendency mentioned here in the case of *Stylidium* is a very common phenomenon in genera which are mainly more

hygrophytic. There seems to be a general tendency throughout the Angiosperms towards the production of such forms—forms which are destined gradually to invade the drier parts of the globe which have hitherto not been colonised.

As stated above, there are four species of *Styloidium* which occur outside the continent of Australia and which thus form marked outliers to the general generic distribution. The range of all four is in a northerly direction from the geographical centre of the genus. The most distant species are *S. Kunthii* in Burma and Indo-China and *S. tenellum* in Burma and the south-east Himalayan region. These two species are entirely extra-Australian. A third species, *S. uliginosum*, is found in north Australia and also in Ceylon and on the southern coast of China. The fourth and last species, *S. alsinoides*, is found in northern Australia and in the Philippines. It will first be noticed that all these species are in connection with the Australian continent across a somewhat close chain of isolated land surfaces, separated by comparatively narrow areas of water. The islands of the Malayan Archipelago form two such chains bridging the distance between Asia and Australia. The westerly of these leads north-westwardly to Burma, the eastern leads northwardly to China. Of the four species under consideration it will be seen that two are found at the extremity of the western chain, one at the extremity of each chain and one half-way along the eastern chain. It is worthy of note that nowhere along these chains are there climatic conditions which by lack of rain would present a barrier to the plants concerned. The present distribution of these species seems to indicate that they have attained their present range by passage along these routes, which have allowed the natural expansion of the genus in such a direction. It must be borne in mind that the present distribution is open to a reverse interpretation, namely that the direction of movement has been towards Australia from the north. This view is, however, entirely opposed to the evidence afforded by the range of the family as a whole, which can only be reasonably explained on the assumption that the group is of southern origin and affinities. A movement of individual species away from the centre of distribution must not be confused with the mass movements or migrations caused by change in climatic and other conditions. The present distribution of *Styloidium*, especially the extra-Australian species is taken to indicate a common feature of plant distribution, namely the gradual expansion of area in progressive genera in the directions possible under existing climatic conditions. A similar feature is seen within the Australian

continent in the gradual slight invasion of the drier parts by increasingly xerophytic species.

The view that the genus *Stylidium* illustrates a type of distribution which is the result of absence of profound climatic and other disturbing factors is also supported by the marked geographical segregation of the various sub-genera and sections. Of the six sub-genera, four are small and two are large. Of the former, one contains the two species which connect south-west and south-east Australia, two are confined to northern Australia and included the extra-continental species and one is confined to the south-west. Of the two larger sub-genera one, containing 26 species, is entirely confined to the south-west. In the remaining sub-genus of 64 species there are twelve sections and of these seven are confined to the south-west. Three more sections are exclusively northern and eastern. This leaves two sections. In the first all seven species are to be found in the south-west, but one of them ranges across the south coast to New South Wales and Tasmania. In the second which has 11 species, six are confined to the south-west and five to the south-east and east.

Stylidium exhibits an astonishing variety of growth forms and many of the species are superficially very like characteristic members of quite different families, as is shown in the two sections *Junceae* and *Saxifragoideae*. There are also several examples of single species which have features peculiar in the family. Such are *S. scandens* with a climbing habit; *S. Barleei*, the only species with dentate leaves; *S. guttatum* and *S. appressum* with two distinct kinds of sub-capitate inflorescences, and the two members of the sub-genus *Forsteropsis* with tiny closely imbricate leaves all over the stems. It is significant that all these are confined to one geographical group, *i.e.* that of the south-west. Another aspect of the same feature is the ease with which, from a taxonomic point of view, the genus can be subdivided into groups which have all the appearance of natural divisions. It remains to be seen how far this, together with information derived from the present distribution, throws light upon the history of the family.

In considering the distribution of a group of plants it is interesting to try to correlate this with the general views held as to the phylogeny and relationship of the different parts of the group. In this connection one or two points are important. First, although it must be accepted that all the members of a series of related types are descended from a single more or less remote ancestor it does not necessarily follow that any existing form is very similar to this ancestor, but only that

the living forms differ from it in varying direction or degree. The ancestral form may persist almost unchanged, but on the other hand it may have been superseded entirely. Again, it is misleading to imagine that the existing types all represent stages through which the present most highly developed type has passed in its phylogeny. The ancestral forms of the most specialised type may all have disappeared and the existing less specialised forms may be the apices of different and distinct lines of descent. This point of view has an important bearing upon the subject of plant distribution. A comparatively primitive form may have attained its present features only very recently while a much more specialised form may have attained its present condition much longer ago and remained unchanged for a comparatively long time. Thus from a distributional aspect a specialised form may be very much older than one showing more primitive characters.

Two types of criteria are available in the elucidation of the comparative phylogeny of a series of genera, namely palaeontological evidence and the application of certain tenets as to the indications afforded by different kinds of characters and the general direction of evolutionary tendencies. In general, it is considered that those families, genera or species which show the most generalised characters and which have the least specialisation are the more primitive. In the case of the Stylidiaceae no palaeontological evidence is available, but from a consideration of floral and other structure the genus *Stylidium* must inevitably be considered the most specialised. Closely related to it but lacking some of its extreme features is the genus *Levenhookia*. *Phyllachne* and *Forstera* show a similar but much smaller degree of specialisation. *Orcostylidium* shows least specialisation of all and is usually considered to be the most primitive type.

Assuming the provisional correctness of these views the distribution shows some remarkable features. The advanced type *Stylidium* has multiplied out of all proportion to the others and, as has been pointed out, comprises 88 per cent. of the whole family. On the other hand, the most primitive and possibly oldest genus is a monotype. At the same time it is noteworthy that the two genera *Phyllachne* and *Forstera* which are presumably older than *Stylidium*, both show widely discontinuous distributions, the former from South America to New Zealand and the latter from New Zealand to Tasmania. A distinction must apparently be drawn between those genera with scantily populated but wide discontinuous ranges and those with thickly populated smaller continuous ranges. It may be contended

that the Asiatic species of *Stylidium* give a discontinuous effect, but as has been shown there is reason to believe that these species are recent emigrants from the Australian centre and are not analogous to the species of *Phyllachne* and *Forstera*.

Finally, despite the absence of fossils which might support the view here expressed, the general taxonomy and distribution of the family seems to point to the fact that during a great part of its history the family has occupied only an unimportant position in the general vegetation but that recently forms have been evolved which contain the necessary factors of success and which have spread and multiplied until the family is now one of the most characteristic of the whole Australian flora. The realisation of the possibility of such a history, with marked differences in degree of development at different periods, is a most important phyto-geographical conception.

CONCLUSION AND SUMMARY

The Stylidiaceae illustrate many interesting phenomena of plant distribution and a study of their geography helps to throw light upon some of the principles involved. The following are some of the more important points:

1. The family shows a discontinuous and disproportionate distribution over two widely isolated land areas. This indicates a previous connection, not necessarily a complete land bridge, between New Zealand and South America across the South Pacific.
2. There is very great difference in the relative development of the different portions of the family, especially the genera. The Australian elements are much the most successful of the family at the present time. This appears to be due, on the one hand to a vigorous vitality and ability to form new species and on the other hand to the fact that the conditions in the region in which they are found nearly approach the optimal developmental conditions of the particular group of plants concerned.
3. The genus *Stylidium* in particular shows, in its distribution, almost complete correlation with the areas in which the annual rainfall averages at least a certain figure and absence of this amount of rain is a definite distributional limiting factor. This factor appears to have no limit on the side of excess.
4. The genus *Stylidium* indicates that the generic area is slowly increasing by the exploitation of suitable new areas by certain species. There is no evidence that the movement is a migratory one involving the whole family.

5. In *Stylidium* there is very marked geographic segregation of the various parts of the genus. Moreover, all the species of the genus show very much the same degree of relationship and there are no conspicuous gaps in the general range of affinity. This is taken to show that the present generic distribution has developed gradually and naturally, unaffected by profound climatic changes or other disturbing factors.

6. The available evidence indicates that *Stylidium* and *Levenhookia* are the most advanced genera of the family, and that *Phyllachne* and *Forstera* are less advanced and possibly of similar age. Hence it is seen that the, presumably, youngest genus has an enormously greater number of species than any of the others.

7. The genus *Oreostylidium* appears to be a primitive form and to be a good example of a monotypic endemic genus which is a relic (epibiotic of Ridley). At the same time it is not to be assumed that the other genera of the family are necessarily descended from *Oreostylidium*-like ancestors but only that *Oreostylidium* is more like the common ancestor than any of the other forms.

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ON THE GENERA *PHYLLOPHORA*, *GYMNOGONGRUS* AND *AHNFELDTIA* AND THEIR PARASITES

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(With 5 figures in the text)

FOR the sake of those who do not make a special study of the algae, or at any rate, the red algae, I propose in this paper to make a critical survey of the present state of our knowledge of the plants named in the title. When I have finished, I think I shall have shown, that as the result principally of the labours of Friedrich Schmitz about thirty years ago, we have reached in the case of nearly all of them, a sort of *impasse* in our knowledge, which challenges inquiry from the new generation of algologists who have succeeded him.

The literature to which I shall mostly refer in due course is included in the four following papers:

- 1 SCHMITZ, FR Die Gattung *Actinococcus* Kutz. *Flora*, Heft 5 1893.
2. GOMONT, M. Note sur le Mémoire récent de M. Fr. Schmitz, intitulé Die Gattung *Actinococcus* Kutz. *Journal de Botanique*, no du 1^{er} Avril. 1894
- 3 DARBISHIRE, O. V Die *Phyllophora*-arten der westlichen Ostsee (Deutscher Antheil); Neue Folge, Band 1. Kiel 1895
- 4 DARBISHIRE, O V On *Actinococcus* and *Phyllophora*. *Annals of Botany*, 13. 1899

Any other papers to which it may be necessary to refer, will be included in a bibliographical list at the end of this paper.

Let us consider in the first place what species are included in the genera *Phyllophora* and *Gymnogongrus*, and which of them are to be found in British waters. For the first purpose let us take De Toni's work on Florideae (1897), and for the second purpose, Holmes and Batters' List of British Algae (1892).

We find that the genus *Phyllophora* was divided by Agardh (1876) into three sub-genera (an arrangement adopted by De Toni): 1. *Coccotylus*, including two species *Brodiaei* and *interrupta*; 2. *Phyllophora*, including three species, *rubens*, *nervosa* and *Heredia*; 3. *Phyllo-*
tylus, including two species, *palmettooides* and *membranifolia*. Of

these seven, one in each sub-genus, viz. *Brodiaei*, *rubens* and *membranifolia*, are readily accessible British species.

The genus *Gymnogongrus* includes about 30 species of which two, viz. *G. Griffithsi* and *G. norvegicus*, are found on the British coasts. The distribution of this genus is very wide—India, Tasmania, the Cape, Peru, Chili, Pacific and Atlantic coasts of North America, Europe and the Mediterranean.

We find that Agardh included these two genera with two others in the sub-order Tylocarpeae of the order Gigartineae. The two other genera are *Stenogramme* and *Ahnfeldtia*, each represented in British waters by one species, respectively *interrupta* and *plicata*. The genus *Ahnfeldtia* is regarded by Schmitz and Hauptfleisch (1897) as one of uncertain position.

Now let us examine the three British species of *Phyllophora*, *Brodiaei*, *rubens* and *membranifolia*, more closely. All three may be found growing together in the same rock-pools on the coast of Anglesey, though I am not suggesting that they have all three precisely the same habitat and tidal range. I have found, for instance, *P. Brodiaei* growing on stones imbedded in sand along the Menai Straits with only the terminal segments of their branches exposed, in places where neither of the other species could be found. Darbishire found all three (with two other species) in the Western Baltic, where he obtained them, of course, only by dredging. He has an admirable description, in the third paper mentioned at the outset, of their external and internal structure, their attachments, and their reproductive organs as he then understood them.

All three grow from a basal holdfast, attached to a solid substratum; all three exhibit a dichotomous branching in one plane, reminding one of *Chondrus crispus*, but *P. Brodiaei* and *P. membranifolia* have cylindrical stalks, while *P. rubens* is flanged to the base and also possesses a perceptible midrib. Their colour is different—*P. membranifolia* is purplish, *P. Brodiaei* is pinkish, and *P. rubens* scarlet.

But it is on the reproductive structures that it is necessary, for the purposes of this paper, to focus attention. To this end it will be convenient to take the case of *P. membranifolia* first. We find that it conforms to the Floridean tradition in occurring in three forms—male, female, and neutral. It is trioeicious, or as we would now say with Svedelius, diplobiontic. In the one biont the sexes are separate, and the other biont is the tetrasporiferous plant. The spermatia arise on spermatophores or specialised leaflets in crypts, reminding one of a

similar condition described by Thuret and Bornet (1878) in *Gracilaria comfervoides*. They are figured by Darbishire, who made out the apical pore of the crypts, not detected by Buffham (1890), who had however seen the spermatia. The carpophores also occur along the edges of the

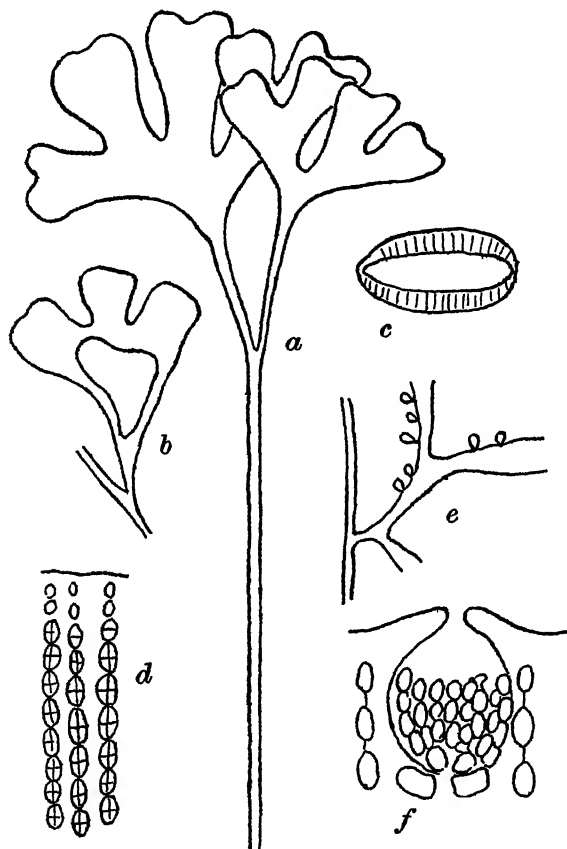


Fig. 1. *Phyllophora membranifolia*.

a, fragment of thallus; b, ditto with nemathecium; c, section through nemathecium; d, threads with tetrasporangia; e, thallus with carpophores; f, antheridial crypt. a orig., b-f from Darbishire.

mature lamina, and by sectioning the young leaflets the structure of the procarys may be observed, and the development of the cystocarp. The tetraspores are produced in nemathecia in broad areas on both sides of the lamina of the neutral plants.

It may be explained here that a nemathecium is a fructification consisting of threads the successive cells of which become transformed into tetrasporangia each giving rise when mature to a set of tetraspores. When these threads lie loosely curved in the interior of the thallus as in *Chondrus crispus*, for example, we may speak of a "medullary nemathecium," but when they arise from the cortical cells and lie closely packed together more or less parallel to one another at or near the surface we may designate the whole structure a "cortical nemathecium." The nemathecia of *P. membranifolia* are cortical nemathecium, as are all those hereafter referred to in this paper.

In respect of the structure of its cystocarp and nemathecium, *P. membranifolia* greatly resembles *Stenogramme interrupta*. In the structure of its cystocarp it resembles also *Chondrus crispus*, but this latter plant has, as I have already mentioned, a medullary nemathecium.

Let us turn next to *P. Brodiaei*. This also has antheridia in crypts like *P. membranifolia*. Buffham does not seem to have found them. Darbishire, however, figures them, and I am able to confirm the accuracy of his drawings. *P. Brodiaei* has also cystocarps produced in much the same situations as in *P. membranifolia*, but they have been only rarely recorded, apparently once at Kiel by Kuckuck, again in material collected at Heligoland, but never yet, so far as I can ascertain, in British waters.

But plentiful fructifications of another kind occur on *P. Brodiaei*, and have found their way into every herbarium of algae. These look like spherical warts on the edges, and usually near the base, of the crop of proliferating leaves so characteristic of this species. Most of the specimens were labelled nemathecium, though it was held that in some cases the tubercular structures were true cystocarps. Schmitz, however, declares that in every case when he came to examine the so-called cystocarps, they turned out to be nemathecoid in structure. Besides, as I have said above, the true cystocarp found so rarely in *P. Brodiaei* has a quite different appearance.

But what about the nemathecoid tubercles themselves? Were they the true nemathecium of *P. Brodiaei*, homologous with the somewhat similar structures in *P. membranifolia*? They had fallen under suspicion as far back as 1819, when Lyngbye declared them to be the fructification, not of *P. Brodiaei* itself, but of a parasitic red alga preying upon it. This view was adopted by Kützinger (1843), and the parasite appeared in the *Phycologia generalis* under the name of

Actinococcus roseus. But until we come to Schmitz's time, algologists do not seem to have adopted this view without reserve. Harvey, for example, in the *Phycologia Britannica* (1849-51) speaks of these structures as the genuine nemathecium of *P. Brodiaei*. The fact that

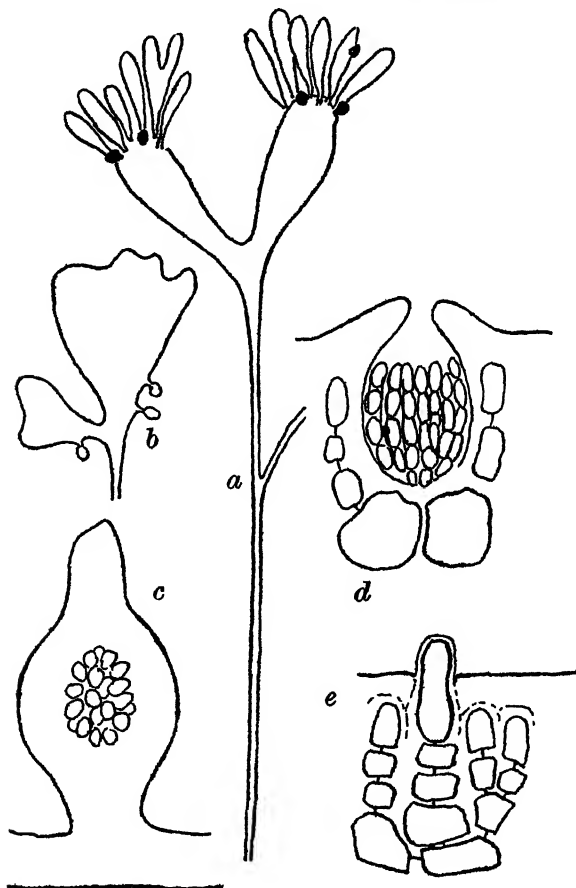


Fig 2 *Phyllophora Brodiaei*.

a, fragment of thallus with pseudo-nemathecium; *b*, thallus with carpophores; *c*, single carpophore; *d*, antheridial crypt; *e*, procarp. *a* orig, *b-e* from Darbshire.

the nemathecium of *P. membranifolia* had not been called in question, and that these tubercles on *P. Brodiaei* were somewhat similar in minute structure, no doubt accounted for the hesitation in accepting the idea of their parasitic nature. Besides, if these were not the true

nemathecia of *P. Brodiaei*, it would be left without any tetrasporic fructification at all. When Schmitz comes to investigate the nature of these tubercles, he makes short work of these considerations, and emphasises what he regards as the indisputable evidence of their parasitic nature afforded by the anatomical investigation. He points out that the similarity in the nemathecia of the parasite, to the undoubted nemathecia of other species of the host-genus, finds its parallel in other cases of parasitism among Florideae. I cite here a list of such cases:

Balbiana on *Batrachospermum*, both Nemalieae;
Gonimophyllum on *Nitophyllum*, both Delesseriaceae;
Janzewskia on *Laurencia*, both Rhodomelaceae;
Stromatocarpus on *Polysiphonia*, both Rhodomelaceae;
Episporium on *Ceramium*, both Ceramiaceae;
Choreonema on *Corallina*, both Corallinaceae;
Callocolax on *Callophyllis*, both Gigartinaceae.

I would be inclined to add:

Harveyella on *Rhodomela*, both Rhodomelaceae;

for though *Harveyella* has been placed by systematists in Gelidiaceae, it has a procarp reminiscent of the Rhodomelaceae, as Sturch points out.

A similar situation is not unknown among Fungi, where *Thamnidium* and *Chaetocladium*, for example, both Mucorini, prey upon *Mucor*. Even among flowering plants, Loranthaceae are parasitic on Loranthaceae in India. The similarity in the nutrition and general metabolism between allied plants no doubt makes the transition to parasitism easy in all these cases.

With reference to the other consideration that *P. Brodiaei* would on the admission that the nemathecioid warts belonged to the parasite itself be without a tetrasporic fructification, that could not be allowed to weigh if the evidence from anatomy as to their parasitic nature were conclusive, and this evidence Schmitz claims to submit in the paper entitled "*Die Gattung Actinococcus*" mentioned above.

Schmitz had previously published in 1889 his "*Übersicht der bisher bekannten Gattungen der Florideen*," in which he admits the validity of *A. roseus*, but without submitting (as was natural in an epitomised list) the grounds on which he based his decision, and which afterwards he gives in detail in the paper of 1893. Reinke (1889) adopted Schmitz's decision of 1889, and also admitted *A. roseus* in a list which he was himself publishing. Later, in 1892, inasmuch as

Schmitz had not in the meantime published anything in justification of his decision as expressed in the "Übersicht," Reinke expressed doubt as to the autonomy of *A. roseus*, and returned to the view that the warts upon *P. Brodiaei* had not been disproved to be the true nemathecia of that plant.

This immediately brought Schmitz into the arena with the paper mentioned in an early paragraph, and if anyone desires to arrive at a judgment upon the findings of Schmitz, it is to this paper that he must turn, with whatever of the material he can gather together for concurrent microscopic examination by the methods advocated by Schmitz. There is no doubt that the anatomical investigation is difficult, and the inexperienced in such exercises may be pardoned for not finding a judgment easy.

It is sufficient to state here that Schmitz declares himself driven to the conclusion that the nemathecioid fructifications on *P. Brodiaei* are those of a parasite, as he had long before suspected, and that the true nemathecia of that plant were yet to be discovered if they existed. With regard to the parasite, that also is short of the male and female phases of an ordinary Floridean, no signs of antheridia or cystocarps having appeared in all his anatomical studies.

Before I deal with the wider sweep of Schmitz's discoveries recorded in this paper, let me briefly refer to the case of *P. rubens*, the third species of *Phyllophora* common in Britain. The spermatophores and carpophores are as well-known in this plant as they are in the case of *P. membranifolia*. The antheridia and spermatia were described by Buffham (1893), but the crypts in which they arise are extraordinarily different from those of the species already described.

The ripe cystocarp is figured by Darbishire and is only distinguished from that of *P. membranifolia* by what looks to be superficial folds which have yet to be accounted for. There is also a nemathecioid fruit, which occurs on the quite short stalk of proliferating superficial shoots of somewhat peltate shape. Sometimes similar shoots arise again on those of the first order, on the stalks of which in turn new nemathecioid growths arise, and so a somewhat complex fructification is formed, quite different from the swollen spherical structure belonging to *P. Brodiaei*. Equally with these, however, Schmitz found them to belong to a parasite, which he was disposed to regard as generically distinct from *Actinococcus*, and to which he gave the name of *Colacolepis incrustans*. The difference between the two parasites calling for this generic separation lay in the fact that

Actinococcus was an endophyte with an intramatrical vegetative part that preyed upon the host, and an extramatrical portion devoted more particularly to reproduction, while *Colacolepis* was an epiphyte formed of an external crust, preying upon its host by means of superficial haustoria.

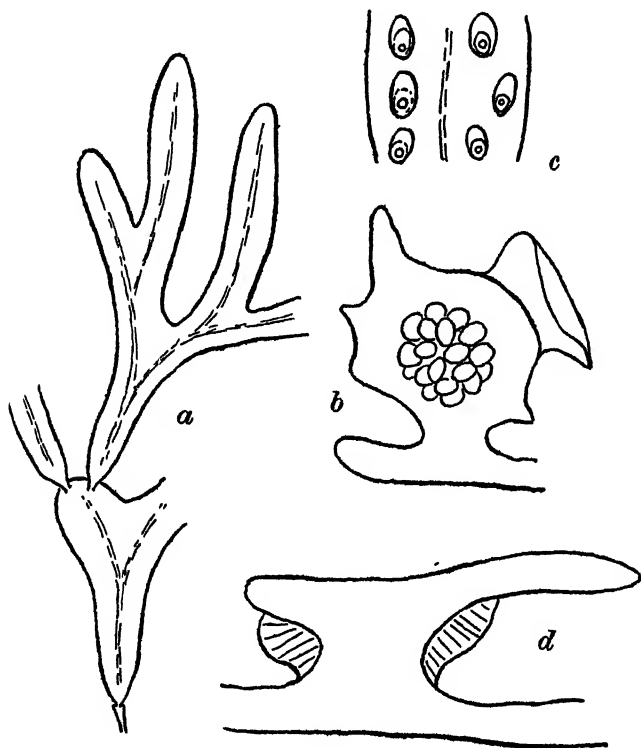


Fig 3 *Phyllophora rubens*.

a, fragment of thallus; *b*, carpophore; *c*, superficial proliferations; *d*, thallus showing position of pseudo-nemathecia. *a* and *c* orig., *b* from Darbshire, *d* from Schmitz.

In this case also the host was left without a tetrasporiferous fructification, and again no sexual phases were found belonging to the parasite.

But, as I have stated, Schmitz's survey took a wider range than the examination of the three species of *Phyllophora*, viz. *P. membranifolia*, *P. Brodiaei*, and *P. rubens*. He extended his observations to *P. palmettoides* and other species of *Phyllophora* falling into the same Agardhian sub-genus *Phyllotylus* with *P. membranifolia*, and

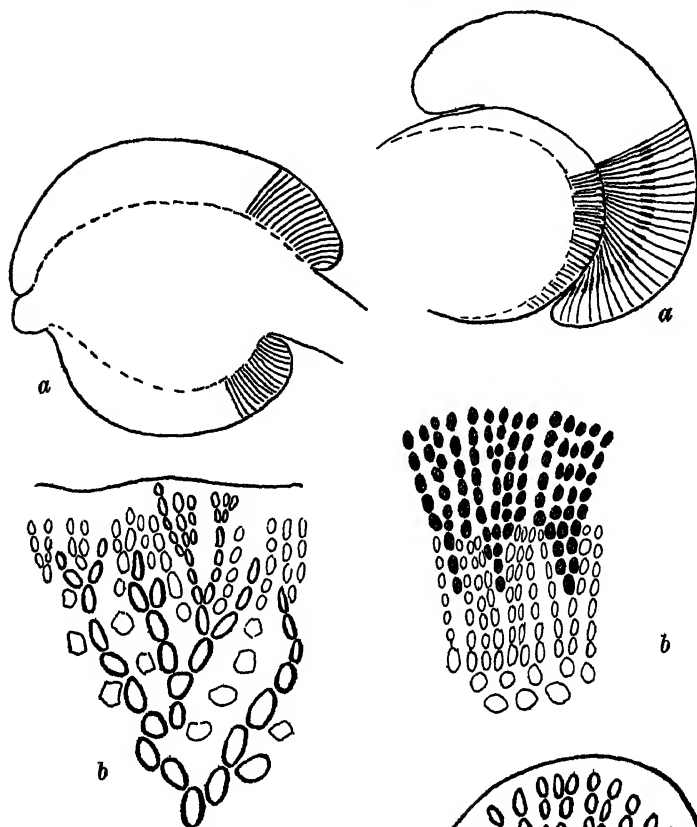


Fig. 4. *Phyllophora Brodiaei*.

a, section of the thallus with two opposite emergences of the parasite *Actinococcus subcutaneus*; *b*, section showing the initiation of an emergence, the difference in the character of the cells exaggerated. After Schmitz.

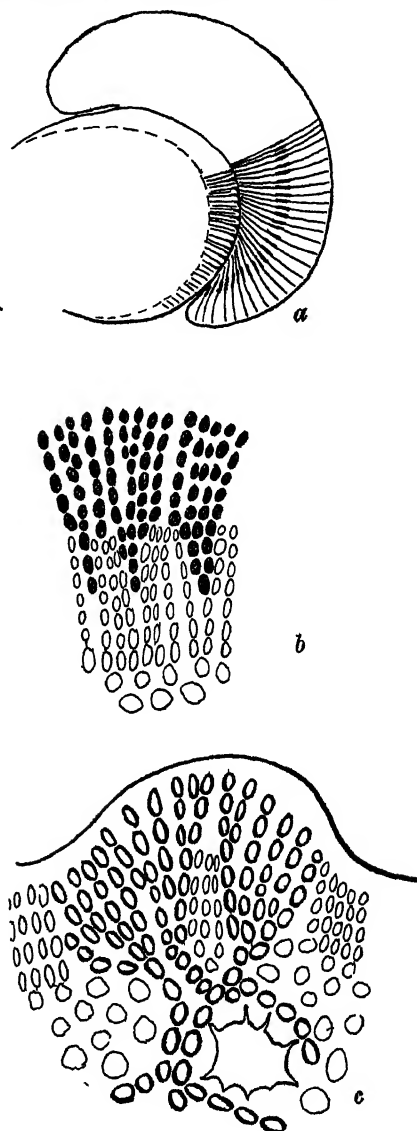


Fig. 5.

a and *b*, *Ahnfeldtia setacea*. *c*, *Gymnogongrus Wulfeni*.

a, section of the thallus showing the epiphyte *Stereocolax decipiens*; *b*, section showing the suckers (sinkers) of the epiphyte (black) growing down among the threads of the host (clear); *c*, section showing the initiation of an emergence of the parasite *Actinococcus aggregatus*, the difference in the appearance of the cells of host and parasite exaggerated. After Schmitz.

found that it agreed with *P. membranifolia* in possessing nemathecia of its own. He examined *P. interrupta*, falling into the same sub-genus *Coccotylus* with *P. Brodiaei*, and found that it also was the prey of the same parasite and possessed no nemathecia of its own. He examined also *P. nervosa* and *P. Heredia*, which fall into the same sub-genus *Phyllophora* with *P. rubens*, and found that they also, like *P. rubens*, were preyed upon by parasites, that on *P. nervosa* being identical with the *Colacolepis incrustans* found on *P. rubens*, that on *P. Heredia* being specifically distinct, and receiving the name of *Colacolepis decipiens*.

At the same time he gave it as his established judgment that in the genera *Gymnogongrus* and *Ahnfeldtia* wherever a nemathecioid fructification was to be found, it was due to similar parasites belonging, as he thought, to the genus *Colacolepis*, or to another genus which he named *Sterrocolax*. This leaves many of the species of the two genera *Gymnogongrus* and *Ahnfeldtia* without reproductive organs of any kind, for, unlike *Phyllophora*, no cystocarps have ever been described for some of them. In *Colacolepis* and *Sterrocolax* equally with *Actinococcus* no reproductive organs have been found other than the nemathecia. So firm is Schmitz in his contention that he predicts that if the rest of the species of *Gymnogongrus* and *Ahnfeldtia* be examined, and as I have indicated already these genera are widely represented, they will be found with a tetrasporiferous parasite, and presumably without any tetrasporiferous fructification of their own.

In order that the remarkable nature of the situation as left by Schmitz may be realised and made clear to the eye, I am presenting the results of his findings in tabular form (see p. 251).

On the left are the hosts with two spaces for the expected Floridean phases, the carposporiferous and the tetrasporiferous. On the right are the parasites (when they occur) with two similar spaces. A cross indicates a recorded occurrence, a dot the absence of any record. The information in the case of the foreign algae in the list is taken from De Toni's *Sylloge Algarum*, Vol. 4 (1897). No attempt is made in the table to indicate in what species antheridia have been observed, but they may at least be assumed to occur in all the cases in which cystocarps are recorded. The star indicates a British species.

It will be observed:

1. That in the three species in which there are no parasites the plants have both cystocarps and tetrasporangia.
2. That in all the cases in which parasites occur, the host, if it possesses any known reproductive structures at all, bears only cystocarps.

3. That every parasite is, on the other hand, devoid so far as is known, of cystocarps, and bears only tetrasporangia, in some cases only monosporangia.

It would seem, therefore, as if the presence of the parasite has a sterilising influence on the host, totally inhibiting the production of tetraspores, and at any rate rendering less frequent the production of carpospores. At the same time the parasite seems itself to have lost its power of sexual reproduction altogether, and has to rely for its propagation upon the tetraspores.

Hosts and parasites of the sub-order Tylocarpeae of the
Order Gigartinales

	Host	♂		♀		Parasite (if any)
		Q	⊕	Q	⊕	
	* <i>Stenogramme interrupta</i>	x	x			
Phyllo- tylus	* <i>Phyllophora membranifolia</i>		x			
	" <i>palmatoides</i>		x			
Cocco- tylus	* " <i>Brodiaei</i>		.	.	.	<i>Actinococcus subcutaneus</i> (=10cous)
	" <i>interrupta</i>	x	.	.	.	" "
Phyllo- phora	* " <i>rubens</i>	x	.	.	.	<i>Colacolepis incrustans</i>
	" <i>nervosa</i>		.	.	.	" "
	" <i>Heredia</i>	x	.	.	x	" <i>decipiens</i>
	<i>Gymnogongrus Wulfenii</i>	" <i>aggregatus</i>
	* " <i>Griffithsia</i>	.	.	.	x	" "
	" <i>vermicularis</i>	.	.	.	x	" <i>peltaeformis</i>
	" <i>patens</i>	.	.	.	x	" "
	* " <i>norvegicus</i>	x	.	.	x	" "
	" <i>dilatatus</i>	.	.	.	x	" <i>laticus</i>
	" <i>crenulatus</i>	.	.	.	x	" <i>peltaeformis</i>
	" <i>fastigiatus</i>	x	.	.	x	<i>Sterrocolax crassior</i>
	<i>Ahnfeldtia setacea</i>	" <i>decipiens</i>
	* " <i>plicata</i>	.	.	.	x	" "

The second paper—that by Gomont—in the list which I have placed at the beginning indicates the reception of these findings of Schmitz by an experienced French algologist. It was in a Paris herbarium that Schmitz found the material of *P. rubens*, *P. nervosa* and *P. Heredia*, which enabled him to decide that the supposed nemathocia of these species were also those of a parasite. Gomont was therefore greatly interested in the results published in Schmitz's paper, and had the curiosity to repeat his investigations as far as possible. To this end he had access to the fine collections of Bornet, now in the laboratory of the Museum of Cryptogamic Botany in the Jardin des Plantes. His researches convinced him of the accuracy of Schmitz's discoveries, and in this conviction he was confirmed by Bornet himself to whom Gomont showed his preparations. Further, he was able to add Farlow's species *P. Clevelandii* to the

membranifolia category as a species with true nemathecium of its own, and *Gymnogongrus linearis*, material of which Schmitz had not been able to obtain, to the species of that genus with a parasite providing the nemathecium. He differs from Schmitz in some details, but closes with the hope that the latter might yet solve the crowd of interesting problems his discoveries had raised and that by the study and culture of the living algae.

In January of 1895, however, Schmitz died suddenly in the prime of life to the great loss of botanical science in many departments, and in particular in that of the Rhodophyceae where he was an acknowledged leader of a new school.

The hopes of Gomont and others that Schmitz might himself do something to solve the new problems were thus doomed to disappointment; for it is difficult to believe that Schmitz with his active mind would have been content to leave so many species with truncated life-histories without strenuous efforts to extend our knowledge of them.

It was not until the summer of 1895 that Darbishire's monograph on the *Phyllophorae* of the Western Baltic was published. In this work, however, the decisions of Schmitz with regard to the parasitic nature of the nemathecium structures of *P. Brodiaei* and *P. rubens* were not accepted. I have already made considerable use of the information supplied in this paper concerning three of the five species of *Phyllophora* with which it deals. It has the great merit of being profusely illustrated with drawings depicting many phases in the life-histories.

Nothing further on the subject was published until 1899 when Darbishire wrote the fourth paper in the list, viz. that on *Actinococcus* and *Phyllophora*. In this the writer handsomely acknowledges that, as the result of further researches of his own, he was now convinced of the parasitic nature of the warts on *P. Brodiaei*. In the interval between the publication of the monograph in 1895 and 1899 he had pursued his investigations at Kiel and was now able to show that the parasite obtained a foothold on the *Phyllophora* by means of the antheridial crypts on the spermatophores. He suggests, moreover, that another route of entry might be the procarpia on the carpophores, though as female plants so very rarely occur, judging from the rarity of the cystocarps, this route cannot often be made use of. I have, however, myself seen what seemed to me to be derelict procarps, as well as in other cases old antheridia, in the neighbourhood of the *Actinococcus* nemathecium.

The whole matter thus rests much as Schmitz left it thirty years ago, and yet it is difficult to believe that our knowledge has reached a stage at which algological science can afford to let it rest.

A glance at the synoptical table on p. 251 shows at once the gaps in our knowledge. To take *P. Brodiaei* and *P. rubens* first. Assuming that *P. Brodiaei* produces carpospores more regularly than we suppose, though as has been said, cystocarps have never been recorded from Britain, and have been only rarely found elsewhere, have both these species dropped out the tetraspore-bearing phase entirely from their life-cycle? And with regard to *Gymnogongrus* and *Ahnfeldtia* it is impossible that so many of them maintain themselves without reproductive cells of their own of some kind, yet for most of them there is no record of either carpospores or tetraspores. Then with regard to the parasites which all seem to be equipped with tetraspores (or perhaps monospores), have they lost all traces of sexual reproduction, as seems to be the case?

Until new facts are forthcoming to aid us in answering these queries, it is surely permissible to suggest possible solutions, if only as working hypotheses. To take the species of *Phyllophora*, for example, one suggestion occurs to one inevitably. Is it possible that their tetraspore stage is after all represented by the parasite? Reinke made this suggestion to Darbishire long ago, but neither seems to have thought the idea worthy of serious consideration. It ought, however, to be capable of being brought to the test of experiment, for in that case the tetraspores of the nemathecium ought, on cultivation, to reproduce the *Phyllophora*. The carposporophyte in diplobiontic Florideae is always parasitic on the gametophyte generation, is it impossible that in these cases the tetrasporophyte is so also? All the anatomical facts which to Schmitz proved the parasitism of the nemathecium-bearing plant do not touch this issue, for on this supposition the carpospore would germinate on the gametophytic host and give rise to the tetrasporiferous stage, as if it were a parasite.

On this hypothesis also it would not then be necessary to look for sexual stages of the parasite. And at any rate no such sexual stages have ever been seen.

With regard to the genera *Gymnogongrus* and *Ahnfeldtia*, while cystocarps are recorded for some species of the former genus no reproductive cells of either kind are recorded for the latter.

Of the species of *Gymnogongrus* producing cystocarps, the British species *G. norvegicus* is one. Assuming that it has ceased to produce tetraspores of its own, and assuming that the sexes are separate, as they generally are in Florideae, two kinds of plants ought to occur,

viz. male plants which would appear to the collector sterile, and female plants which would ultimately bear the cystocarps. Now the nemathecia of the parasite ought to occur indifferently on both these forms. Careful examination ought to discover whether this is so.

And although *Ahnfeldtia* is regarded by Schmitz and Hauptfleisch as of uncertain position, it has at any rate according to Schmitz a parasite belonging to the same genus as that of *G. fastigiatus* and probably stands near to that genus. Is it impossible to find out how the British species *A. plicata* propagates itself, although it is recorded for all fourteen of the sections into which Holmes and Batters (1892) divided up the British coast for algological purposes? The reproductive cells described by Buffham (1893) are almost certainly monospores from the nemathecia of the parasite, and not spermatia as at first suggested by him.

Now how is our knowledge of these algae likely to be most effectively extended? I would answer first, by extending our knowledge of the biology of at any rate the British species, secondly, by extending our researches into the anatomy of fresh or suitably preserved material; and thirdly, by means of cultures under continuous observation either in the laboratory or in the open.

With reference to the first point I mean by it the close observation of accessible living plants at all seasons of the year, and in variable habitats. In Schmitz's list on p. 251 I have starred the British species. There are also in British waters two other species of *Phyllophora*, viz. *P. palmettoides* and *P. Traillii*. The latter is figured by Batters (1889) and appears to be equipped with cystocarps but no nemathecia. All these British species require to be better known from the biological point of view.

On the second point, their anatomical investigation has been by no means exhausted. A good deal of Schmitz's work had to be done on herbarium material, and though the tough texture of these plants is better preserved in the dried condition than that of more delicate algae, yet dried plants are an inadequate substitute for fresh or appropriately preserved material. Schmitz does not seem to have seen, for example, in all his careful anatomical investigations the antheridia described by Darbishire, and found by him to be the path of entry of the parasite, at any rate Schmitz makes no reference to them so far as I know. In the case of *P. Brodiaei* there are also curiously grape-like colourless tubercles ("Traubenkörper") to which reference has been made both by Schmitz and Darbishire. Schmitz thought they might be due to still another species of *Actinococcus*. Darbishire speaks of remains of antheridia and procarps on

them together with tetraspores. They certainly demand further investigation.

On the third point, the investigation of these plants in seaside laboratories by means of cultures, I would lay great stress. It is true that they will be found slow-growing, and results must not be expected with these perennial plants to come as quickly as with annuals. In the case of *P. Brodiaei*, it might be found possible to transfer adult plants growing on stones into controlled tanks, after having removed all tubercles by excision before they are mature, and then to see if in a new season they would still produce tubercles. The infection is said to be quite local, and if so, tubercles ought not to appear on the new crop of shoots where neither tetraspores nor carpospores can reach them. Then there is the cultivation of tetraspores and carpospores under control. An excellent beginning in this field was made by Darbishire who kept germinating tetraspores of *A. subcutaneus* (= *roseus*) under observation for two years in the belief at the time that they were the true tetraspores of *P. Brodiaei*. His figures of these germings are suggestive, and show, at any rate, that the spores are amenable to cultivation.

Observations such as these may prove difficult and tedious, but it is not easy to see how further advance in our knowledge can be made except by resort to some such laborious and long-continued research. On the other hand, some data may be found which will clear up the situation more quickly than we anticipate.

I am indebted to Miss A. J. Davey, M.Sc., for her kindness in copying for me, from the original sources, the figures accompanying this paper.

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REVIEW

Bibliographia Genetica I, 1925—(three papers). *Resumptio Genetica* (Deel I, Afl. 1 and 2). Ed. J. P. LORSY and H. N. KOOIMAN. Published by Martinus Nijhoff, The Hague.

As genetics is probably the most widely dispersed section of biological science it is scarcely surprising that an attempt should at last be made to compile an adequate summary of its literature. This new venture is ambitious in that it deals with the past as well as the future. All genetic literature published before the end of 1923 is to be concentrated and reviewed in a number of articles which will together constitute *Bibliographia Genetica*; of these Valentin Haacker's *Aufgaben und Ergebnisse der Phänogenetik* (pp. 220), Heilborn's "Genetic Cytology and Genetics in *Carex*" (pp. 5), and Punnett's "*Lathyrus odoratus*" (pp. 13) have been received. There can be no question that they are of great value; it will now be possible for students and research workers entering the subject to get into touch with the work of the past in a far shorter space of time than heretofore and further to obtain—to use a military metaphor—an appreciation of the situation in 1924 by a staff officer of the first rank.

Genetic literature of 1924 and after is to be summarised in *Resumptio Genetica* of which two parts of the first volume are to hand. To each part there is a prefatory list of new publications arranged in four groups dealing with general, anthropological and medical, zoological, and botanical and agricultural papers respectively. The reviews themselves are arranged under six headings; it is suggested that this further subdivision may be a little unfortunate. The separation of papers on breeding-work in flax, the production of new varieties of potatoes and barley and their degree of resistance to diseases, etc. under a separate agricultural heading is not likely to be of help to geneticists as a whole and further it is doubtful if agricultural workers are likely to gain anything by the award of this position of "splendid isolation."

The first part of vol. 1 enumerates about 270 papers and has reviews of about 30; the second enumerates about 300 and reviews about 170, so that if all the papers mentioned are to be reviewed there is leeway to be made up. There are one or two instances of papers reviewed whose titles are not present in the bibliographical list, which is unfortunate. The majority of the botanical reviews are concise and give a clear picture of the content of the papers concerned; it is perhaps only to be expected that reviewers have in almost all cases contented themselves with summarising the work and offer no criticism as to the matter or the validity of the conclusions. The range of papers is very wide, extending from studies of the embryo sac in *Senecio* to sexuality in *Coprinus*, and from the systematics of *Oenothera* to the genetics of plant form in mosses; this is all to the good as it must serve to emphasize the breadth of the base on which future genetic theory must stand. There is a good appreciation of Correns in connection with the *Gesammelte Abhandlungen* and two long reviews of Wettstein's recent work on mosses. The majority of the notices are in German, a fair number in English, and regrettably few in French.

The publication is in the hands of Martinus Nijhoff and is attractively put together; unfortunately the type-setting is frequently at fault and a little more care in proof revision would have avoided many faults which if not material are at least irritating.

S. M. W.

THE NEW PHYTOLOGIST

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THE EFFECT OF ARTIFICIAL AERATION OF THE SOIL ON *IMPATIENS BALSAMINA* L.

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(With 7 figures in the text)

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INTRODUCTION

ARKER (1900) observed that the rate of elongation of roots of *Lupinus albus* and *Helianthus annuus* may be increased by drawing air through the soil in which these plants are growing, and a greater development of their root systems can be secured by altering the soil texture so as to reduce the resistance offered to the natural movement of the soil air. Hunter (1912) showed that the development of the root systems of young plants of *Helianthus annuus*, *Pisum sativum*, *Triticum sativum* and *Lepidium sativum* depended on the texture of the soil in which they grew, and that the development of the subaerial portions of the plants was indirectly determined in this way. On drawing 15 litres of air daily through the soil of pots containing poorly-developed seedlings of *Pisum sativum* with weak stems and curled leaves, it was noted that the seedlings became more robust than those plants not subjected to this treatment—their leaves became larger and their stems stronger.

Hall, Brenchley and Underwood (1914) found that better root development and better growth resulted with silver sand or with

kaolin, than with fine sand, silt, or in water culture. On growing barley in culture solutions aerated once a day, and continuously, a much better root development and a better leaf and stem development were obtained by continuous aeration.

Livingston and Frec (1917) noted that after replacing soil air by nitrogen the absorption of water by the roots ceased within 24 hours in *Heliotropium peruvianum* and *Coleus blumei*.

Bergman (1920) investigated the behaviour of various land plants whose roots were submerged in water, and concluded that such symptoms as wilting, etiolation, and the loss of leaves resulted from a decrease of the absorbing power of the roots. This is also indicated by the corresponding fall of transpiration which occurs. Aeration of the water enabled the plants selected to endure these abnormal conditions without the adverse effects which otherwise appeared.

Turpin (1920) suggested that increased plant activity (growth) is accompanied by increased carbon-dioxide production. This is supported by the fact that a relationship was shown to exist between the carbon-dioxide produced in the soil, presumably by the crop, and the amount of water transpired.

Knight (1924) found that maize in soil-culture showed an increase in dry weight if the soil is aerated, but failed to respond to aeration in a nutrient solution. Wallflowers and *Chenopodium album* showed considerable increase in dry weight as the result of aeration of the culture solution.

Clements (1921) has summarised the known facts concerning the rôle of oxygen in root activity so that a more complete historical account of the literature relevant to the present investigation is rendered unnecessary.

Although the general beneficial effects of artificial aeration of the soil in which certain plants are grown have been recorded, few data are available of a more detailed character concerning the effect of this treatment on any particular plant. In this communication an account is given of the effects of artificial aeration on the development of *Impatiens balsamina* (a greenhouse annual). The reason for the selection of this plant was that preliminary experiments made by one of us, which have not been reported previously, showed that it was extremely sensitive to the degree of aeration of the soil. The experiments of Bergman also indicated that this plant would prove suitable for the present investigation.

All the plants used were grown in a mixture consisting of sixteen parts of fresh turf loam, eight parts of leaf mould, and one part of

sand. Aeration was effected by the operation of a filter pump which was connected suitably to the containers of the soil in which the plants were growing. The amount of air drawn through the soil was determined from the readings of a calibrated flow gauge which was inserted between the pump and the containers. The soil air was removed from beneath the root systems so that, as it was withdrawn, it would be replaced by atmospheric air from above the soil surface containing a smaller proportion of carbon-dioxide (Russell and Appleyard, 1915).

GENERAL DEVELOPMENT

In order to ascertain the effect of artificial aeration of the soil on the general development of *Impatiens balsamina*, series of experiments were conducted at different periods of the year. Seedlings, carefully selected so as to be as uniform as possible with regard to their initial condition of development and vigour, were transplanted into 10-inch glazed stoneware pots provided with tubulures. A length of lead tubing, coiled in a horizontal plane and with thirty perforations at uniform intervals, was installed on the bottom of each pot. One end of the coil of lead tubing in each pot was passed through the tubulure to the exterior, the other extremity of each coil within the pot being closed. Except for the protruding lead tubing each tubulure was then sealed so as to render it air-tight. Crocks were arranged in each pot to a height of 2 inches above the lead tubing, then a layer of leaf mould, above which was placed the soil. The soil air could be changed by connecting the free ends of the lead tubes to the filter pump and causing this to operate. Three seedlings were planted in each pot and ten pots were employed, so that the development of thirty plants was recorded during each period of experiment. No artificial aeration of the soil took place until the seedling plants had become thoroughly established after being transplanted. After this was accomplished 3 litres of air were drawn each day through the soil of pots numbered 1, 2, 3 and 4; 6 litres of air were drawn daily through the soil of pots 5 and 6; while pots 7, 8, 9 and 10 served for control purposes and the soil in them was not aerated artificially. The three periods during which observations were made on the development of the plants were from November 1st, 1921, to December 10th, 1921; from January 25th, 1922, to March 15th, 1922; and from April 20th, 1922, to May 27th, 1922.

The rates of development of the subaerial portions of the plants were compared by means of the weekly percentage increases in the surface areas of their leaves and stems. These were calculated from

measurements taken with a sliding calliper accurate to 0.01 mm. The results obtained are presented in Table I.

Table I

Average percentage increments in surface areas of leaves and stems of plants of *Impatiens balsamina* recorded at successive stages in their development. (a) Average increments in Pots 7, 8, 9 and 10, which were not artificially aerated; (b) increments of plants in Pots 1, 2, 3 and 4, through the soil of each of which 3 litres of air were drawn per day; (c) the increments of plants in Pots 5 and 6, through the soil of which 6 litres of air were drawn per day.

Interval	Leaves			Stems		
	Aerated			Aerated		
	Not aerated Pots 7, 8, 9, 10 (a)	Pots 1, 2, 3, 4, 3 litres per day (b)	Pots 5, 6, 6 litres per day (c)	Not aerated Pots 7, 8, 9, 10 (a)	Pots 1, 2, 3, 4, 3 litres per day (b)	Pots 5, 6, 6 litres per day (c)
Nov. 1-Nov. 11	5.73	4.365	5.3	0.29	0.37	0.52
" 1- " 18	6.76	8.392	9.031	0.92	1.04	1.26
" 1- " 25	8.79	10.667	10.65	1.29	1.63	1.65
" 1-Dec. 2	10.12	11.472	12.23	1.52	2.00	2.06
" 1- " 10	11.02	14.497	14.29	1.55	2.02	2.33
Jan. 25-Feb. 1	1.407	1.445	1.506	0.07	0.255	0.13
" 25- " 7	2.60	3.20	3.51	0.33	0.49	0.54
" 25- " 14	4.41	4.78	6.18	0.44	0.82	0.85
" 25- " 22	7.58	8.34	9.01	0.97	1.45	1.55
" 25-Mar. 1	10.46	11.71	13.20	1.39	1.90	1.99
" 25- " 8	14.15	16.29	18.53	2.17	2.83	3.03
" 25- " 15	20.95	24.83	27.67	2.82	3.65	3.89
Apr. 20-Apr. 28	3.22	3.62	4.28	0.411	0.476	0.531
" 20-May 5	10.37	13.24	16.35	0.884	0.993	1.092
" 20- " 12	34.71	46.9	49.45	3.115	3.051	4.005
" 20- " 20	61.04	80.12	84.35	5.505	5.61	6.485
" 20- " 27	89.4	173.2	150.4	9.4	11.9	11.7

It will be noted that the effect of artificial aeration of the soil could always be detected by this method owing to the larger increases in the surface areas of both stems and leaves which were evident after this treatment had been in operation for eighteen days, and which usually became apparent after a much shorter period. The increases of the surface areas were not uniform during the months of November-December, but, in the January to March period, and in the April to May period, they were more regular (see Fig. 1). The irregular increases during November and December were probably due to the unsuitable conditions for growth which prevail at

that time of the year. On analysis of the data obtained it was found that distinct "grand periods of growth" could be recognised about four weeks after the seedling *Impatiens* had been replanted. The "grand period for growth" did not arrive earlier as the result of the artificial aeration of the soil, but the maximum points reached were higher in the case of the treated plants than in those of the control plants, or the growth at the more rapid rate was maintained by the

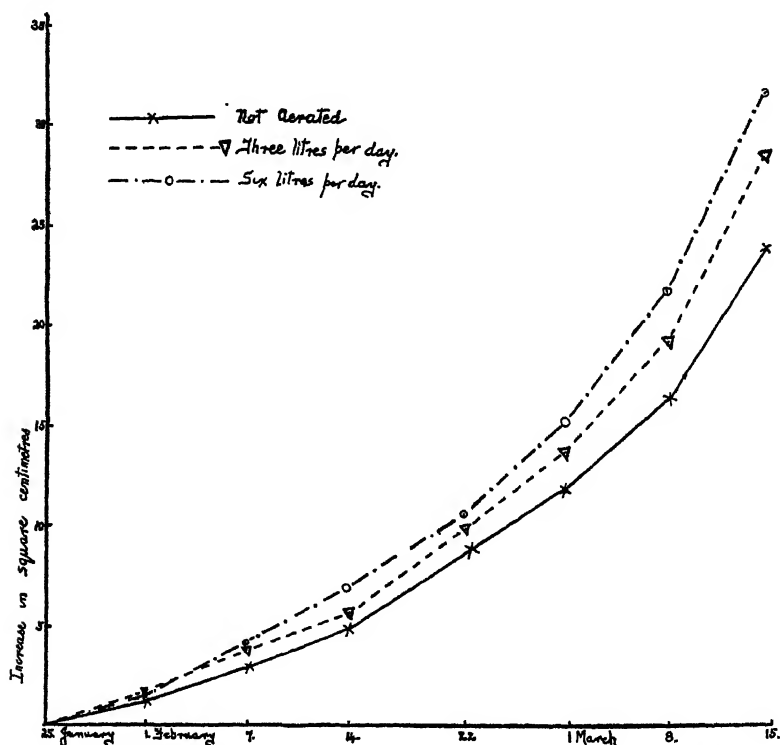


Fig. 1. Effect of aeration on the rate of increase of surface area of leaves and stems of *Impatiens*.

treated plants for several days longer. Examination of the data shows that the beneficial effects of the artificial aeration of the soil on the development of the subaerial portions of the plants are not in direct proportion to the amount of air drawn through the soil. Although the development of those plants in pots through which 6 litres of air were drawn daily was generally better than that of the plants in pots through the soil of which only 3 litres were drawn,

this may merely result from the more thorough removal of the soil air. A comparison of the total development attained by the entire plants in the artificially aerated soil and the naturally aerated soil at the end of each culture period (see Table II) gave results which were in agreement with those recorded above.

Table II

Increased development of plants grown in artificially aerated soil taking the plants in the Pots 7, 8, 9 and 10 (not artificially aerated) as the standard.

	November-December period		January-March period		April-May period	
	Pots 1-4	Pots 5-6	Pots 1-4	Pots 5-6	Pots 1-4	Pots 5-6
Average increase of	%	%	%	%	%	%
Length of root	65	45	16	47	12	12
Leaf surface	21	17	24	27	93	68
Stem surface	27	31	18	27	13	16
Fresh weight	26	26	33	27	17	12
Dry weight	31	31	—	—	9	8

RATE OF GROWTH

In addition to the variations which occur in the rate of growth of a plant such as *Impatiens balsamina* according to the regular daily alternations of light and darkness, there are also minor fluctuations which occur during both the daytime and the night. Artificial aeration of the soil caused an alteration in the occurrence of these fluctuations. The rate of root elongation was recorded by observations made on the roots of plants growing in a compartment one side of which consisted of a sheet of glass suitably inclined. Measurements were made by means of horizontal microscopes, precautions being taken to prevent unnecessary disturbance due to the action of light. The periods during which elongation of the root proceeded without interruption varied considerably, but usually they were about 20 minutes in duration. Each period of active elongation was succeeded by an interval during which little or no elongation took place. These interruptions of elongation were not of constant duration but, in the case of an active plant, they usually lasted from 5 to 10 minutes. The normal elongation of the root of *Impatiens balsamina* therefore does not proceed at a constant and uniform rate, but periods of active growth are separated by intervals during which no elongation takes place. The irregular duration of the periods of active growth and of the intervals during which no elongation takes place would seem to indicate that this activity is dependent on a

complex of variable factors. Artificial aeration of the soil causes the elimination of the intervals during which root elongation ceases under ordinary conditions and, after this treatment, the elongation of the roots proceeds until a much longer interval has elapsed. Thus, on January 26th, the periods of elongation were from 15 to 18 minutes in duration before artificial aeration of the soil, but, after

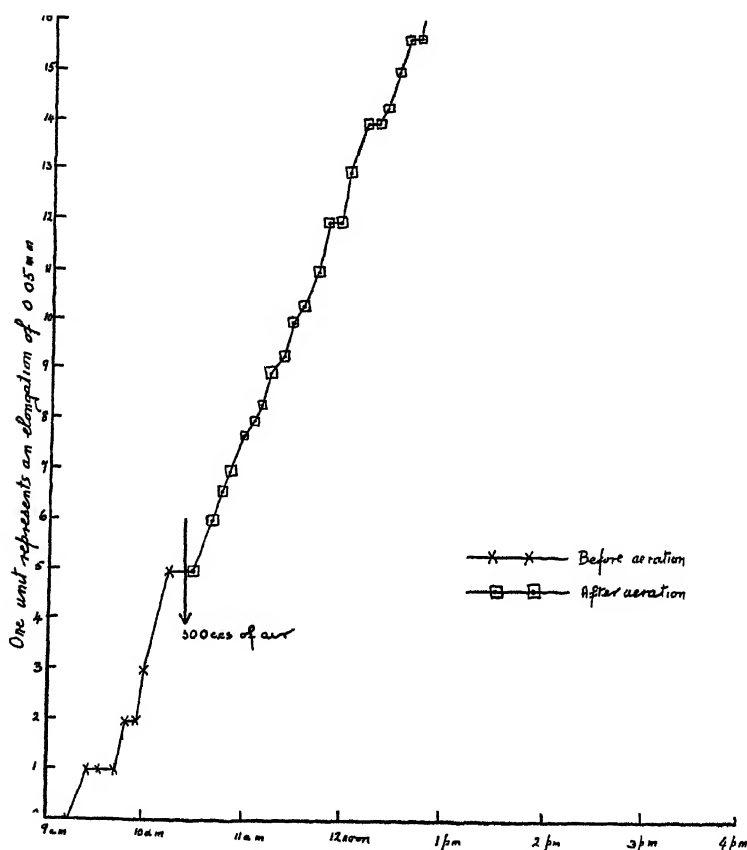


Fig. 2. Effect of aeration on the rate of elongation of the root of *Impatiens*

drawing 500 c.c. of air through the soil, the elongation of the root proceeded for a period of 80 minutes without interruption. On February 7th the periods when growth was interrupted occurred at intervals of from 10 minutes to 20 minutes, but after the soil had been artificially aerated the root elongated without interruption for 1 hour 20 minutes. From a large number of similar observations it

must be assumed that the temporary disappearance of the intervals during which elongation of the root is interrupted is a direct and immediate consequence of replacing the soil air by atmospheric air (see Fig. 2).

Further evidence in support of this is afforded by the fact that roots which had failed to increase in length during abnormally long intervals of time, or whose rate of elongation was extremely slow, resumed active elongation after artificial aeration of the soil (see Fig. 3). Also, an increased rate of elongation always occurred as a consequence of artificial aeration of the soil.

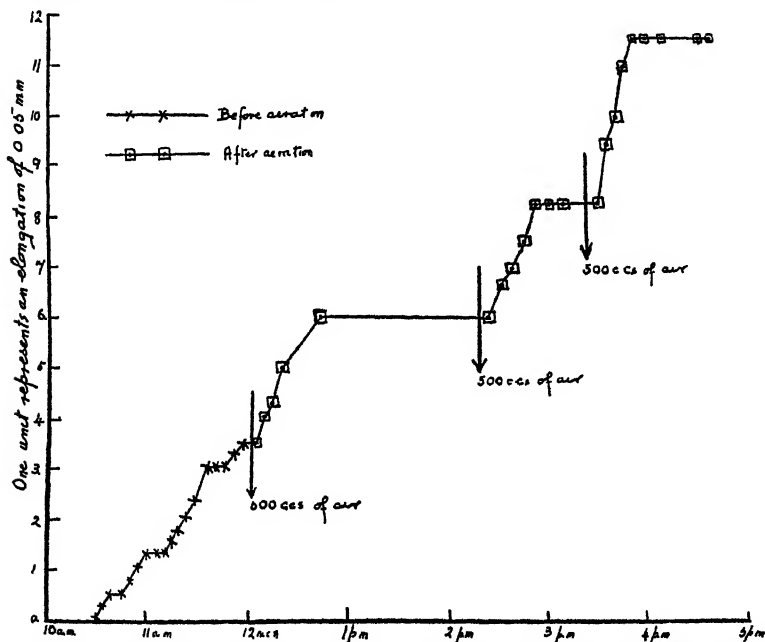


Fig. 3. Effect of aeration on the rate of elongation of the root of *Impatiens*.

The normal elongation of the stem, like that of the root, is irregular and interrupted by periods when growth is either very slow or temporarily ceases. In view of this it was considered necessary to determine whether these alterations in the rate of elongation of the stem were in any way related to those which had been observed for the roots. By the use of an apparatus devised by us (Hunter and Rich, 1923) the actual rate of elongation of the stem could be measured directly. The periods of elongation and the intervals during which elongation ceased or was extremely small were found

to be of a similar order for both the roots and stems of *Impatiens balsamina*, but, in some cases, stem growth was taking place so rapidly that no definite rest periods could be detected. After artificial aeration of the soil the intervals of slow elongation, or of absence of elongation, of the stem disappeared, with the result that the rate

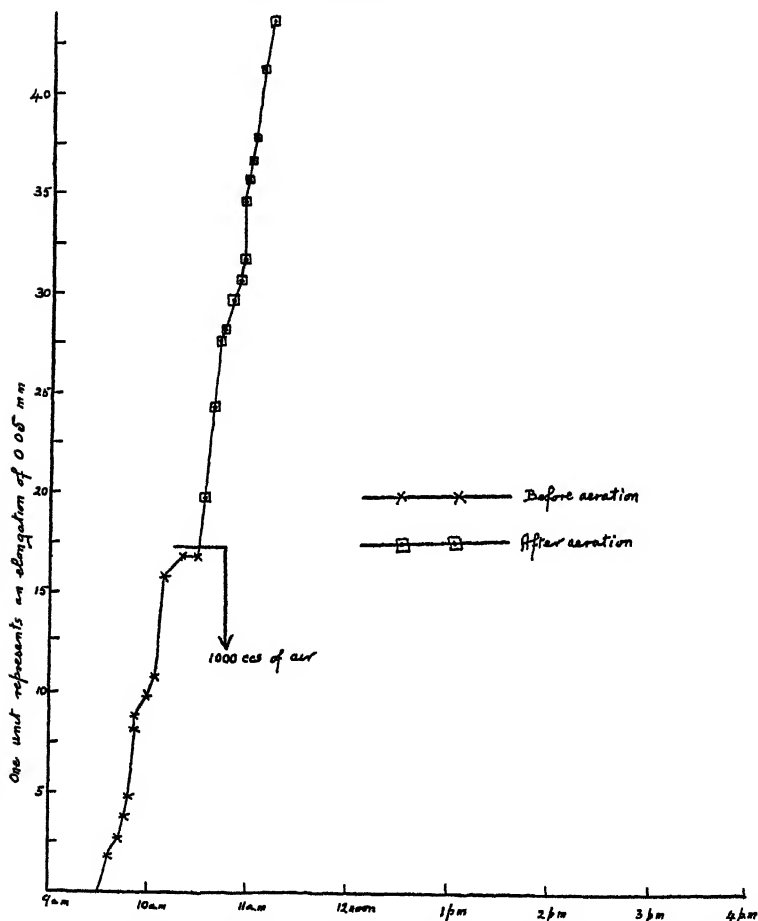


Fig. 4. Effect of aeration on the rate of elongation of the stem of *Impatiens*.

of growth of the stem became more uniform owing to the absence of interruptions (see Fig. 4). Moreover, the actual rate of elongation of the stem is increased by this treatment. These effects are similar to those noted for root elongation except that the more rapid rate of stem elongation continued for a longer period after artificial

aeration of the soil. Stem elongation persisted for about 3 hours, whereas that of the root continued for about 80 minutes after similar treatment. Active growth of the stems of those plants which had not elongated appreciably for some considerable time could also be induced by the artificial aeration of the soil about their root systems. These effects of the action of artificial aeration of the soil on the elongation of both the stem and the roots of *Impatiens balsamina* can be detected at once after this treatment has taken place.

TRANSPIRATION

The method adopted for the estimation of the rate of transpiration was that suggested by Hoffmanns (1912). Four steel springs were used to suspend the plant of *Impatiens balsamina* growing in a wide glass tube, the lower end of which was closed by a waxed cork through which was inserted a length of narrow glass tubing for the additional aeration of the soil. The upper surface of the tube was closed during the periods when observations were taken by means of oiled silk so as to avoid loss of water by evaporation from the soil surface. Artificial aeration of the soil always resulted in an increase in the rate of transpiration. The effect of artificial aeration of the soil on the transpiration current of *Impatiens balsamina* was investigated by means of the pinometer (Darbishire, 1905). This is a continuous piece of apparatus inserted between the shoot and the root and designed to establish the actual relationship existing between root pressure and shoot suction. Any fluctuations in these two forces are recorded by the movements of mercury in a gauge which is attached to the system. The plants used in this series of experiments were grown in ordinary flower pots which had been previously soaked in paraffin wax so as to prevent any penetration of air through their walls. A small coil of lead tubing perforated at intervals and closed at one end was inserted in the base of the pot, the free end of the tubing protruding through the drainage holes, which were otherwise sealed so as to render them air-tight. Crocks and soil were arranged in the pots in the usual manner. Additional aeration of the soil could then be effected by connecting the protruding open end of the lead tubing to the pump.

These experiments were made during the month of October, 1922, and it was found in each case that the force exerted by leaf pull was distinctly greater than that due to the pressure of the sap exuded from the basal portion of the severed plant. The movement of the surface of the mercury in the manometer attached to the system

during fixed units of time served to indicate the balance that existed between leaf pull and sap pressure. In every case the downward movement of the mercury in the outer arm of the manometer was retarded when the soil was artificially aerated, and resumed its normal course after this treatment had been discontinued. This would seem to indicate that the amount of water exuded by the basal portion of the plant was increased as the result of the treatment; consequently experiments were made to determine if this were the case.

The pinometer apparatus was again employed for this purpose, but the upper shoot portion of the plant was replaced by a glass rod. It was found that aeration of the soil caused a distinct increase of the sap exuded as indicated by the movement of the mercury level in the attached manometer, but that after this treatment was discontinued the rate of sap exudation returned approximately to its previous condition.

RESPIRATION

The rate of respiration of the shoot of *Impatiens balsamina* has been investigated by enclosing it in an air-tight glass vessel, the access of light being prevented, and a stream of air free from carbon-dioxide drawn through the vessel. The amount of carbon-dioxide present in the air leaving the vessel was estimated by the usual laboratory methods. It was found that the amount of carbon-dioxide freed by the shoot under these conditions during the daytime but in absence of light diminished until a minimum was reached, after which it continued to increase. As the result of artificial aeration of the soil the decrease of the rate at which carbon-dioxide was freed could be arrested and the rate augmented until a maximum was reached, after which it diminished again until it reached its normal rate, which was then resumed. This interference with the normal respiratory conditions usually continued for about 3 hours (see Figs. 5 and 6).

GERMINATION

The effect of the presence of excess carbon-dioxide and of an insufficient supply of oxygen on the germination of seeds has been recorded by Nabokich (1904), Becquerel (1907), Kidd (1914) and others. Under these conditions it has been shown that the germination of seeds is inhibited or delayed. The effect of artificial aeration of the soil on the germination of seeds of *Impatiens balsamina* was determined in a series of experiments during the months of January

to April (the recognised time for sowing Balsam seeds is in March). Forty seeds were used in each case. These were sown in suitable

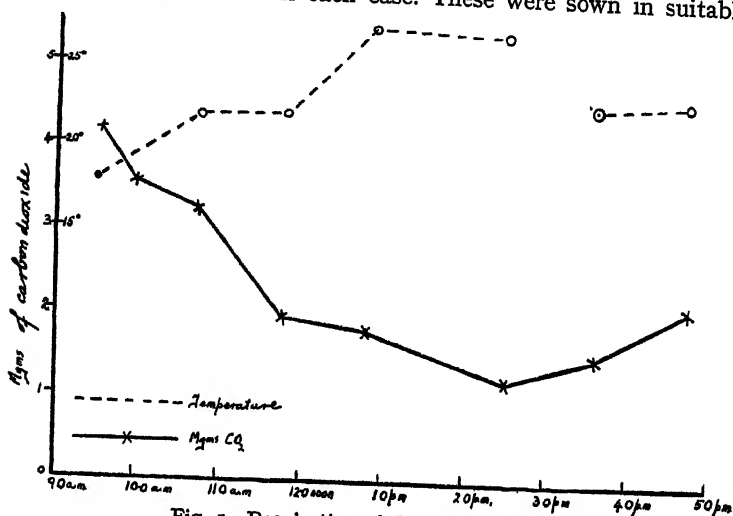


Fig. 5. Respiration of the shoot of *Impatiens*.

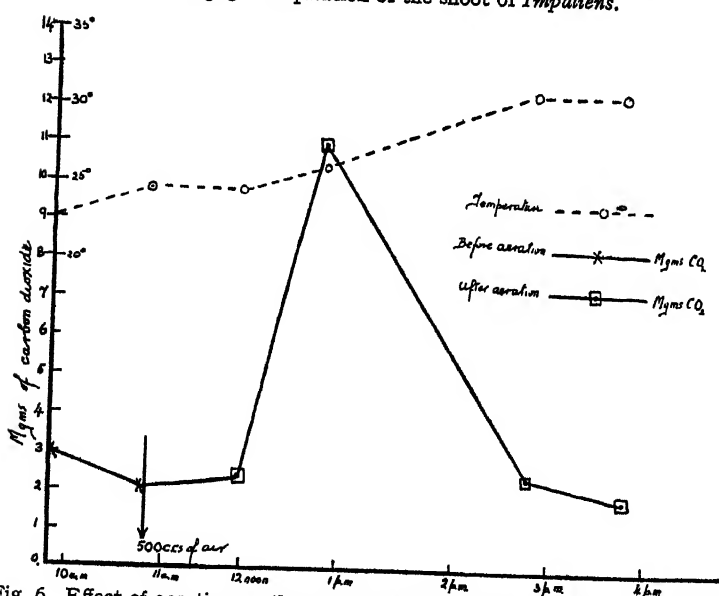


Fig. 6. Effect of aeration on the rate of respiration of the shoot of *Impatiens*.

receptacles, the soil of which could be aerated in the usual manner by withdrawing the soil gases and replacing them by atmospheric

air. Germination of seeds in the artificially aerated soil took place earlier and the total number of seedlings developed was greater than in the soil which was only aerated naturally during the months of January, February and March (see Fig. 7). In April the total number of seedlings developed was not affected, but only the speed of germination. The seedlings in the artificially aerated soil were always better developed than those under normal conditions.

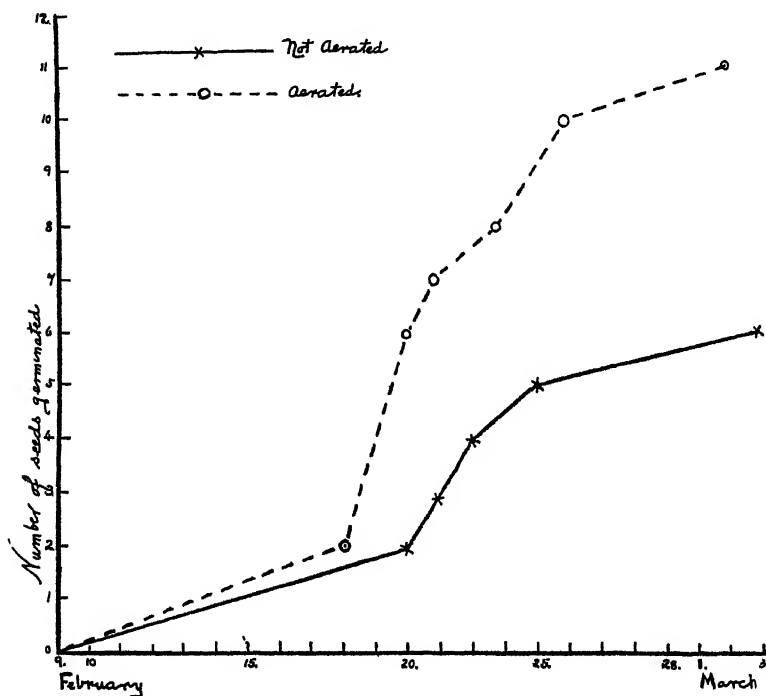


Fig. 7. Effect of aeration on the germination of seeds of *Impatiens*

SUMMARY AND DISCUSSION

The beneficial effects of the artificial aeration of the soil on the *Impatiens balsamina* have been established, and an immediate alteration in the rates of growth, as manifested by more regular and more rapid stem and root elongation, has been detected as a consequence of this treatment. Moreover, the rate of transpiration and the intensity of the respiratory activity of the shoot are augmented by additional aeration of the soil about the root system.

It is suggested that these phenomena are due to the removal of carbon-dioxide in the soil resulting from the respiration of roots and

the activities of the soil organisms. The local concentration of carbon-dioxide around an actively growing root is probably very high. When this concentration has reached a certain intensity it may have a narcotic effect on the protoplasm of the root hairs which are most exposed to its influence. More internal cells of the root would be affected indirectly by the fluctuations of the activities of the root hairs. The functional activities of the root hair cells, and other cells of the root, may be temporarily suspended until such time as the carbon-dioxide accumulation has been sufficiently reduced by natural diffusion, and thus the environmental conditions would permit of the resumption of the normal activities. The rate of absorption of water might be caused to fluctuate owing to alterations in the permeability of the cell membranes occasioned by changes in the intensity of carbon-dioxide concentration in the neighbourhood of the root hairs. The rate of elongation is governed by the degree of turgor pressure in the individual cells. The rate of the absorption of water is one of the limiting factors in its determination. It is feasible to conceive that increased root activity following a diminution of carbon-dioxide concentration after soil aeration would tend to develop a higher degree of turgor pressure in the living cells of the shoot and thus influence the rates of transpiration, growth, and respiration of this portion of the plant. As has been recorded above, it was found possible to detect and measure increases of each of these activities. Further experimental evidence is necessary before this theory of the mode of action of artificial aeration on plant activities can be established fully; but it is considered advisable to present the results which have been obtained to date.

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LIGHT AND GROWTH

I. THE EFFECT OF BRIEF LIGHT EXPOSURE UPON ETIOLATED PLANTS

By J. H. PRIESTLEY

(With Plates VII-X and 1 figure in the text)

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INTRODUCTION

WHEN a flowering plant is grown in darkness, remarkable structural and morphological changes frequently result, which are spoken of as the effects of etiolation. These changes have been variously regarded as due to the "stimulus of darkness" (Noll(7)), to the indirect results of the absence of light, as in the view that they are due to defective nutrition (Jost(4)), or as due to the absence of the directly formative influence of the light upon the growing plant. (These earlier theories are very fully reviewed in MacDougal's comprehensive monograph(5)).

Throughout the extensive literature of etiolation, however, there are to be found few records of observations upon the effect of light upon previously etiolated plants, although such observations can alone test the soundness of suggestions as to the formative influence of light, and an early observation of Batalin(1) seems to point the way. He found that etiolated leaves developed to a considerable size, even in darkness, if previously exposed for $1\frac{1}{2}$ to 3 hours to a light, too faint to permit them to turn green during such a short exposure.

It is true that Ricôme(9) has published the results of a long series of observations upon the development of etiolated plants when transferred to normal daylight, but his method of experiment does not provide much elucidation of the problem. Such etiolated plants are very apt to succumb if placed suddenly in strong sunlight, possibly owing to excessive transpiration from the succulent tissues, which are protected only by a very thin cuticle.

But if conditions are not too extreme and they survive, then the plant, as it grows on, reverts to normal structure in its newly formed tissue, though Ricôme notes that the first stem internodes formed in the light may be shorter and stouter, and the first leaves formed may have a larger lamina than usual.

But how light causes the production of tissues of normal form and structure in a previously etiolated plant, and the intensity and duration of light necessary for these changes, are questions upon which further information is greatly needed. On this subject Trumpf(11) has recently published an important paper, and his results will now be given somewhat fully, and supplemented by experimental data obtained in the Botanical Laboratory at Leeds, as these experiments seem calculated to throw considerable light upon the phenomena of etiolation.

THE EXPERIMENTS OF TRUMPF

Trumpf determined to employ artificial light of definite intensity, and used nitrogen-filled lamps of the Osram Co., which, photo-metrically tested, proved to be of 60 H.K. (Hefner candle power). By the use of white enamel reflectors the light from the lamps was raised to an intensity of 90 H.K. at one metre distance and the lamps were always used in pairs, one on either side of the plant at some 25-30 cm. distance. For higher light intensities the arc lamp of a Zeiss epidiascope was employed, and was placed on one side of the plant, but to avoid unilateral effects the plants were kept revolving in front of the light by means of a klinostat.

The intensity of this light could be varied by altering the current strength and by the use of a reflector. Running water was used to cut down the heat radiation. In preliminary experiments various plants were used and finally *Phaseolus multiflorus* chosen for the main series of experiments. Individual plants are very variable in growth rate, but so far as possible uniform material was obtained by germinating a large stock of seedlings for from seven to eight days in the dark and then selecting from this material plants with uniform root length. An effort was made to cut down temperature differences between plants grown in the dark continuously and those under temporary light exposures, by placing the dark chamber just above the lamps, but the temperature of the plants in the light certainly rose a degree or two above that of the control.

In the first experiments, with two lamps with a light intensity about 2880 M.K. (metre candle), the control plants in the dark were compared with others which had respectively half-hour, two-hour, four-hour and twelve-hour exposures to light daily. The plants lit, even for the shortest period, showed a change from the normal etiolation form and dimensions, internodes becoming shorter and leaf lamina larger; there is no indication at all of a limiting intensity or duration of light, below which no effect can be produced. The plants with four-hour and twelve-hour light exposure showed considerable development of chlorophyll.

Another series of plants were now grown at higher light intensities (40,000 M.K.) but with shorter exposures, viz. one minute, five minutes, ten minutes and thirty minutes daily, the plants being placed 70 centimetres away from the light.

The plants grown under these conditions resembled very closely the series with longer light duration, but with this very important difference that the plants with longest daily exposure (ten and thirty minutes), although having as well developed laminas as those previously exposed for four and twelve hours respectively, showed *no development of chlorophyll*. Thus, in the changes of form produced by light on the etiolated shoot, the *quantity* of light is the factor concerned and its action is not produced indirectly through the effect of the products of photosynthesis.

A number of other experiments by Trumpf, in which varying intensities and duration of light were compared, only serve to emphasize this important conclusion with which the Leeds experiments will be found to be in complete agreement.

An interesting point made by Trumpf, is the observation that

if plants of *Phaseolus* with well developed yellow leaves are placed in daylight, they take a long time (seven to eight days) to become green. But if such a lamina is cut off the plant and placed in 5 per cent. cane sugar in daylight, green colour is well developed in two days. Trumpf's conclusion is that when the lamina is still in connection with the growing plant, the available food supply is being utilised in growth instead of in chlorophyll production.

Comparisons of the effect of light radiation of different wavelengths are very difficult. Trumpf tried to make his conditions comparable in the following manner. Coloured solutions (lithium carmine for red, ammonia and copper sulphate for blue) were used in double-walled glass jars, and the solutions to be used were placed side by side in a window, over vessels containing the solution used in Eder's method of determining the actinometric activity of light (this depends on the gravimetric determination of the precipitate of Hg_2Cl_2 thrown down from a solution of HgCl_2 and ammonium oxalate under the action of light); another double-walled bell jar containing only clear water was also placed in the window at the same time. When it was found, for instance, that the actinometric effect of the light passing through the clear water was four times as great as that passing through the blue solution, the effects of short exposure to light of etiolated plants beneath those bell jars were next compared, four lamps being placed outside the blue jar, one lamp only at the same distance from the clear jar. Similar comparisons were made between white light and red light, and thus the comparative effectiveness of red and blue light in modifying the form of an etiolated plant roughly estimated.

Trumpf's tentative conclusion is that blue light seems very markedly to hinder (or rather fails to induce) lamina expansion, whilst red light favours it, but that blue to some extent and red still more favour the elongation of the stem.

Trumpf also tried the effect of exposure to light during periods when growth of the plant was not taking place, either because the plant was cooled to $3-4^\circ\text{C}$., or because of its partial anaesthesia by the introduction of a non-lethal dose of chloroform vapour.

These experiments showed clearly that under these conditions the light still produced its normal effect upon the succeeding growth form, although this effect could not be shown until subsequent growth had taken place *in darkness* at normal temperatures or after the effect of the anaesthetic had disappeared.

One further interesting point in this connection: with a four-hour

period of lateral illumination at normal temperatures, phototropic curvature began to be visible; with a similar light exposure at low temperatures, no phototropic curvature was produced when growth was resumed, but the effect for these exposures upon the forms of the plants was identical.

Trumpf also tried the effect of darkening, by means of tinfoil, portions of the plant, the lamina or epicotyl alone, or part of the epicotyl only, during the period in which the plant was exposed to light.

The result of these experiments was to show that the action of the light was somewhat strictly localised to that region upon which it fell and that if expansion of the lamina took place, there would be little indication of correlative changes in the length of the epicotyl; the length and form of the epicotyl depended directly upon the extent to which the light had access to it.

These experiments of Trumpf will not be discussed until an account of the Leeds experiments has been given. These were in the main carried out during the sessions 1921-2, 1922-3 and 1923-4, photographs and pressed specimens of the plants being exhibited in Section K at the Toronto meeting of the British Association (1924).

Trumpf's work first appeared in accessible form in March, 1924, as his dissertation on the same subject published at Hamburg in 1921 has never been available. In any case, although some of the Leeds experiments cover the same ground, they have been carried out quite independently, and whilst in some respects they are only confirmatory of Trumpf's conclusions, they supplement these in some directions and also emphasize certain general considerations which do not at first sight emerge from Trumpf's experiments

THE LEEDS EXPERIMENTS

These began as the result of some incidental observations made by Mr G. Redington in the course of some experiments (as yet unpublished) on the effect of varying daily periods of light exposure upon sclerenchyma formation. In the course of this work, root stocks of *Pelargonium* and of *Polygonum cuspidatum* were divided, planted in different pots, and grown either continuously in the dark or exposed to daylight each day for periods of two or five hours respectively.

The plants thus obtained are shown in Plate VII, and it is remarkable to note how completely the plants exposed for only two hours daily have lost all traces of the peculiar structural features associated

with growth in darkness; they have, if anything, more extended leaf laminae, and less lengthy stem internodes than the plants given five hours' light daily. At the same time, these plants showed clearly a point that is just indicated in the photograph; the plants with two hours' light a day had very slight traces of chlorophyll, whilst the five hours' exposure to light produced a plant much paler green than the normal.

At this time (1921) Coupin⁽²⁾ had recently published experimental work indicating that etiolation growth features might be due to the absence of products of photosynthesis from these plants (see p. 281), so that the indications supplied by this experiment, that the form changes might be independent of light durations permitting chlorophyll formation, seemed worth following up. As a result, during 1922-3, Miss Laura Parkin carried out extensive experiments in which plants were grown under cardboard boxes, which were lined inside and out by black cloth. The light-tightness of these boxes seemed perfectly adequate, as no actinometric effect could be detected in acidified solutions of potassium iodide or in sensitised photographic paper as used in exposure meters, when these were left under such boxes in the greenhouse for twenty-four hours. Seedlings were then grown under these boxes, and about midday each day, the boxes over certain plants were raised for periods of one hour, four minutes, two minutes and one minute respectively.

The result of one typical experiment with *Vicia Faba* is illustrated in Plate VIII; photographs of similar series are available for *Pisum sativum* also, and there can be no doubt that for these species the striking results shown are quite typical.

The most noticeable result has always been the marked expansion of leaf lamina and disappearance of the plumular hook in plants exposed to daylight for such daily periods as one to two minutes. This observation immediately brought the control plant into criticism. It is true that this plant has been kept practically continuously in the dark, but the average botanical attitude towards etiolation experiments has resembled that of the man accused of continually digging up his seeds to see how they were growing! The occasional removal of the etiolated plant from darkness to examine its development is normal routine. In the light of these experiments with such short time exposures, the question arises as to whether the true etiolated plant is to be seen amongst the plants on Plate VIII, or for that matter whether it is ever seen in etiolation experiments as normally conducted.

With the help of Miss Sarah Ford the experiments were therefore transferred in 1923-4 to a light-tight cellar. Within the cellar a series of cupboards were constructed around the walls, which were again very carefully protected from stray light radiation by thick wooden partitions and substantial black curtains in front, both floor and roof of each compartment being of stone. The only general lighting in the cellar was a low-power electric ruby lamp which could have been employed with safety close to photographic plates of ordinary sensitiveness. The plants were then grown from seed within these compartments and only taken out for examination when the cellar door was closed and the ruby light the only source of illumination.

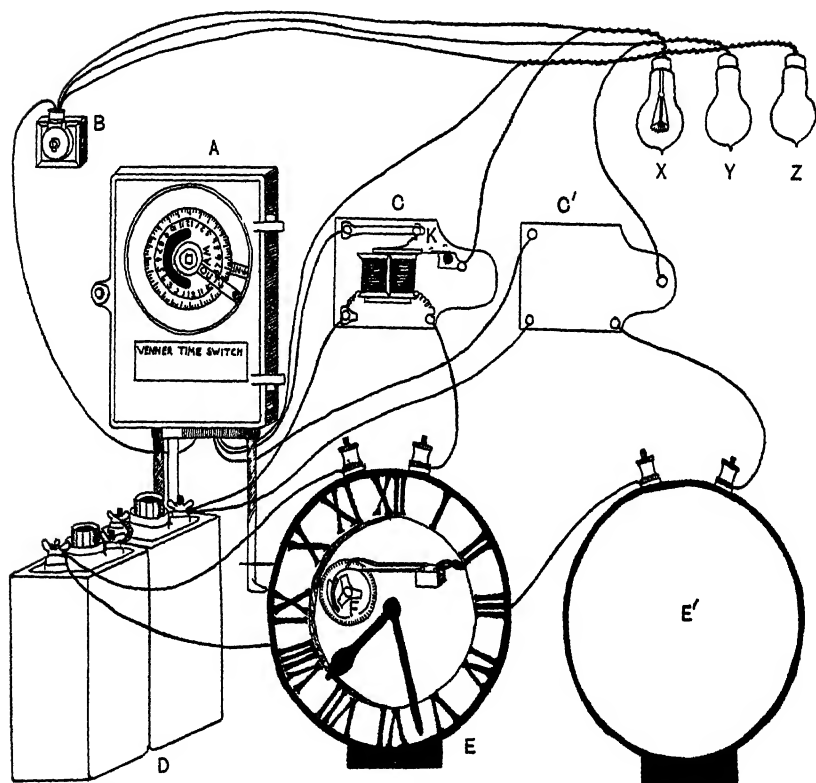
Intermittent lighting of some of the plants was then provided by controlled daily exposures to the light of a $\frac{1}{2}$ -watt nitrogen-filled lamp which proved to have a candle-power of about 80 in the vertical direction; this was mounted on the stone roof of the compartment so that the plants as they grew moved from about 70 cm. to 30 cm. from the light source. Three such lamps were thus mounted in different compartments, the electric circuit to each lamp being controlled by a Venner time switch (kindly lent by Prof. V. H. Blackman, F.R.S., from the Department of Plant Physiology, Imperial College); this switch made the lighting current accessible to each lamp for a period of one hour each twenty-four hours. But two of the circuits were broken again and could only be closed when the current from a secondary battery in a relay circuit actuated an electro-magnet, adapted from an ordinary electric bell fitting; this closure of the circuit depended upon a clockwork device which the Synchronome Co. supplied as a fitting to an eight-day clock, and which permitted of contact being made for a period, adjustable from one to fifteen minutes, of each complete revolution of the hour hands. These periods were adjusted in one circuit at two minutes, in the other at ten minutes, so that though these contacts were made every hour, as the lighting current to both circuits was controlled by the Venner time switch the relay circuit could only send current through the lamps, for periods of two and ten minutes respectively, once in each twenty-four hours. (The arrangement is shown diagrammatically in Text-fig. 1.)

Thus plants were grown under four different conditions:

- (1) Exposed to light for one hour each day.
- (2) Exposed to light for ten minutes each day.
- (3) Exposed to light for two minutes each day.

- (4) In continuous darkness and only exposed to weak red light for occasional inspection.

Plants thus grown are shown in Plates IX and X.



Text-fig. 1. Diagram of apparatus for lighting plants for definite time periods. A Venner Time Switch controls circuit to light Z from wall plug B. Also circuits through C and C', which are closed (at K) for a few minutes each hour by relay circuits from battery D through clocks E and E' respectively. By device shown at F (clock E), length of time current runs through relay is controlled. Circuits through C and C' light lamps X and Y respectively.

Examination of these photographs will bring out certain striking features. In the first place the plants of *Vicia Faba* and *Pisum sativum* grown in complete darkness are now very markedly more etiolated than those shown previously.

In fact it is clear that so long as plants are taken out from darkness to daylight or even moderately strong artificial light for occasional examination, fully etiolated plants cannot be obtained, at

any rate with very light-sensitive plants. Another feature which now becomes very clear in some of the photographs is the marked shortening of the internode which is produced by longer light exposure. In these experiments the plants exposed to light for one hour daily always developed a little chlorophyll.

Whilst the completely etiolated plants retained the plumular hook throughout growth and showed no signs of lateral leaf development, the plants with two minutes' light exposure without exception lost the plumular hook, the apical leaves opened out a little, and the leaves opened out at the second or even the third node. With ten minutes' light exposure the leaves of the plants appeared to be slightly larger than on those plants exposed to light for one hour daily.

If plants from any of the chambers with intermittent lighting were transferred to another chamber in which they were kept completely dark, then in the course of a week or so the plumular hook was again to be seen at the apex and further apical growth was of the type characteristic of complete etiolation, although of course leaves already opening away from the apex might continue to grow for some time.

Structural comparison between the plants grown under these different conditions will be left for a subsequent paper.

DISCUSSION

The Typically Etiolated Plant. The first point to be emphasized emerges more clearly from the Leeds experiments than from those of Trumpf. This is possibly because of the plant he has chosen for experiment; the plumule of *Phaseolus* always shows slightly more development of the first leaves upon the epicotyl than do the plumules of *Vicia Faba* or *Pisum sativum*. Structurally also, it has previously been shown that the effects of etiolation are not so clearly marked in *Phaseolus* as in these other plants(8). Furthermore, it does not appear from Trumpf's account of his experiments that any special precautions were taken against momentary illumination of the etiolated plants during occasional inspection, though it is possible, of course, that they were never inspected until the close of the experiment.

The photographs shown in this paper, particularly a comparison of the broad beans grown in darkness in Plates VIII and X, will make it clear that quite exceptional precautions are necessary if completely etiolated plants are to be produced.

This is true of experiments with such plants as *Vicia* and *Pisum*, but it is only after experimental examination that the point can be determined for any other plant. Thus in the course of the work one plant was experimented with which was far less sensitive to the effect of light. Seedlings of *Lens esculenta* were developed under light-tight cardboard boxes and exposed to daylight each day for periods varying from sixteen to one minute per day. After a fortnight of such intermittent illumination, all these seedlings still retained the same appearance as that of the seedlings only momentarily exposed to light when occasionally examined. In certain cases, the angle between the plumular hook and the axis had changed from being very acute to being slightly obtuse, but this change bore no relation to the amount of light received, and appeared rather to be correlated with the height and therefore the degree of development of the seedling. A repetition of the observation upon lentils using artificial light and all precaution, showed that here also the plants exposed to light, even for two minutes daily, were definitely more developed than those completely etiolated, but the changes in form are not so striking.

Incidentally lentils are the seedlings about which Coupin(3) recently made the absurd statement that the etiolated seedlings grew horizontally, so that apparently if the seeds are planted in the soil the slender etiolated shoots will do their best to girdle the earth instead of coming up into the light. If true, this would provide an interesting problem to the teleologist, but the statement is of course incorrect and, as pointed out elsewhere, probably another instance of an observer misled owing to the great sensitiveness of etiolated seedlings to certain atmospheric impurities (13).

The proof provided by these experiments, that etiolated plants do not obtain their typical development unless the light exclusion is very effective and continuously maintained, throws a flood of light upon certain old controversies which occupy much space in the literature of etiolation.

To cite only two instances. One case previously referred to(8) is the size to which the leaf of certain plants will develop in darkness. Working with the same species of plants, one observer will report development of the lamina to the normal size, another observer will deny any effective expansion of the lamina. The controversy as to fact is now completely explicable; leaves that fail altogether to develop if kept in complete darkness will develop to their full size if the shoot bearing them has received only very occasional or very

weak illumination. In the light of these facts, the old statements in the literature will be found completely reconcilable when the details supplied by the experiments as to their methods are considered.

The other instance that may be taken has recently been exhaustively re-examined by Teodoresco (10).

Briefly stated, MacDougal (5) and Newcombe (6), amongst other workers, denied to a number of climbing plants the power to continue to twine around a support if grown in darkness. On the other hand, Teodoresco, as the result of a number of new experiments, and after a full analysis of the literature, reaffirms the capacity of many of these same plants to continue to twine in darkness.

It is only necessary, however, to re-examine the experimental conditions under which for instance MacDougal and Teodoresco grew their plants to realise that both observers are correct and that the conclusion is that whilst completely etiolated plants fail to twine, plants which are even very faintly illuminated continue to do so.

In this way, future experimental work, in which precautions are taken to prevent the typically etiolated plants ever receiving effective illumination, even during their occasional inspection, will remove many obscurities as to facts. MacDougal many years earlier pointed out (*loc. cit.* p. 283) that the neglect of careful control of stray light in Sachs's etiolation experiments probably accounted for the fact that many of the experimental data recorded by Sachs had not been confirmed by later workers, and took such careful precautions himself that Teodoresco, repeating his observations under less light-tight conditions, fails to confirm them.

The Nature of the Light Effect. The question may now be asked as to whether the experiments of Trumpf and the comparable results obtained at Leeds elucidate at all the manner in which the light affects the growth and development of the shoot. In the first place, these experiments seem conclusive in ruling out the view occasionally advanced, and for which Coupin brought forward experimental evidence, that the formative effects of light are produced through the effect of products of photosynthesis.

Trumpf (12) and Priestley and Ewing (8) have independently repeated the experiments of Coupin and shown that critically examined they will not adequately support the contentions of their author. But the fact that a light exposure of one or two minutes per diem, to a relatively weak artificial light, will effectively remove the most characteristic morphological features of etiolation whilst failing to produce any signs of chlorophyll in the expanded leaf lamina, seems

completely to dispose of the contention that photosynthetic products are concerned in these morphological changes.

Trumpf's experiments enable one to go a little further. The light effects cannot be exerted directly upon the machinery of growth, because if the plant is exposed to light for a brief period in which growth is suspended owing to low temperatures or the action of anaesthetics, when growth is resumed in darkness these morphological changes still result, being brought about by the previous light exposure.

While the results do not, perhaps, rule out a directly "stimulative" effect upon the living protoplasm, whatever that may mean, they do render it necessary to apply such a hypothesis with very great caution. In the meantime, the very short periods of illumination that are effective in some plants point towards some directly photocatalytic change, a type of change which would not be affected by low temperatures during light exposure and which, if it were taking place upon the products of metabolism and not upon the metabolic machinery, would also occur in the presence of anaesthetics.

The evidence thus far, then, is in accordance with some such catalytic action of light, as has been suggested in a previous paper, viz. a photocatalytic action upon fatty or lipid substances which has the result either of releasing them from the surface of the protoplast into the wall or setting them free from combination within the wall, with the result that they slowly diffuse through the aqueous substratum of the cellulose wall and finally accumulate at the surface of the shoot in the cuticle. The way in which such movements of fatty substance might affect the development of the shoot meristem and thus produce morphological changes has already been briefly indicated, and the validity of this theory will be examined in subsequent papers in this series. It will at least be seen that this general theory of etiolation is in accordance with the experimental facts presented in this paper.

SUMMARY

1. Experiments by Trumpf, and in the Botanical Laboratory at Leeds, are described, which serve to show that in certain species etiolated plants are very sensitive to daily exposures of light of only a few minutes duration, marked morphological changes in the shoot appearing as a result.

2. In the light of this observation many contradictions in the literature of etiolation upon questions of fact are readily elucidated.



Fig 1 *Polygonum cuspidatum*

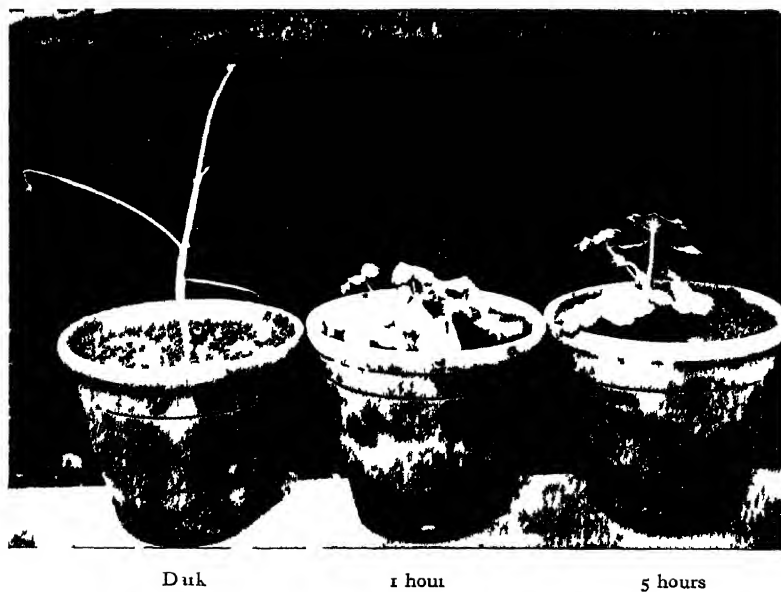


Fig. 2 *Pelargonium*



60 minute

10 minutes

2 minutes

Completely
etiolated

Fig 1 *Pisum sativum*



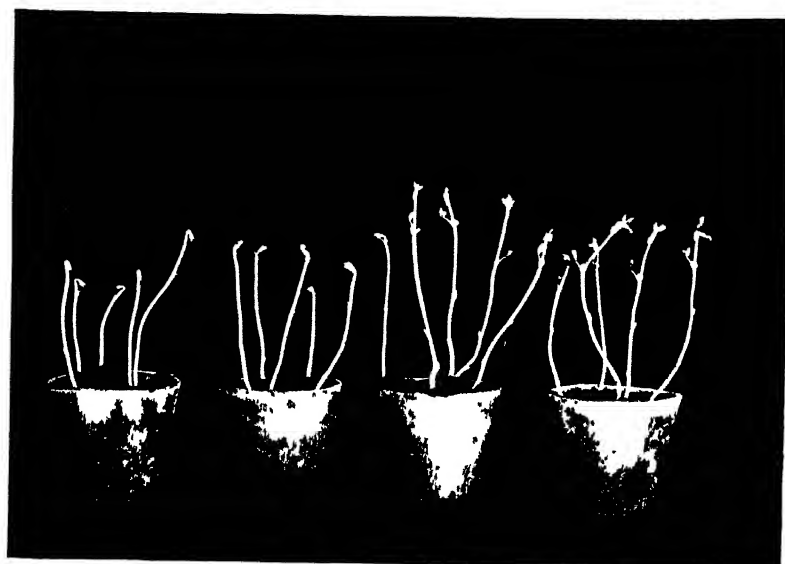
60 minutes

10 minutes

2 minutes

Completely
etiolated

Fig 2 *Vicia Faba L.*



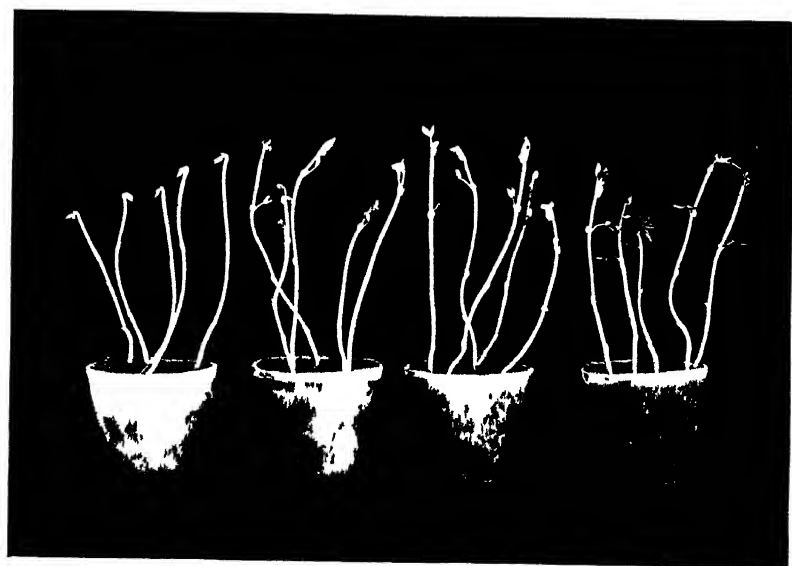
Completely
etiolated

2 minutes

10 minutes

60 minutes

Fig. 1. Later stage than Plate IX, Fig 1



Completely
etiolated

2 minutes

10 minutes

60 minutes

Fig. 2. Later stage than Fig. 1

Pisum sativum. Daily exposures

3. Any explanation of the morphological changes produced upon etiolated plants by light which is based upon the effect of photo-synthetic products is also eliminated.

4. It is pointed out that the light action would appear to be photocatalytic and produced upon products of metabolism in the shoot, not upon the living metabolic machinery itself.

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ON THE ANATOMY OF *OROBANCHE HEDERAE* DUBY, AND ITS ATTACHMENT TO THE HOST¹

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(With 3 figures in the text)

ACCORDING to Hooker(5) six species of *Orobanche* occur in the British Isles. He regards *Orobanche hederæ*, which is parasitic on Ivy (*Hedera helix*), as a sub-species of *Orobanche minor*. Benth(2) states that many species parasitic on different hosts may be only modifications of one species occasioned by the difference of the hosts. The present work deals entirely with *Orobanche hederæ*, which is very abundant in the grounds of University College, Cork, where the work was carried out.

There is much confusion in the literature dealing with *Orobanche*. Beck(1) and Cowles(4) say that contact with a suitable host is a necessary condition for the germination of the seeds; Kerner(6) says they germinate on the ground and further development is dependent on the presence of the host; and Kohler(7) says the seeds germinate at various times whether or not they are covered with soil. According to Beck, *O. hederæ* grows only on Ivy, while Kohler states that it also grows on Conyza and Pelargonium. It is generally agreed that, on germination, the embryo, consisting of an undifferentiated mass of cells embedded in the endosperm, grows out into a delicate filament, which pursues a spiral course through the ground until it meets a suitable host root, into which its apex penetrates until it reaches the xylem; the part immediately outside the host root then enlarges and the upper part of the filament decays (Fig. 1, A, B, C). The body of the parasite then grows larger forming a tubercle, from which are given off short processes which soon cease to grow (Fig. 1, D). Solms-Laubach(12), Kohler(7), and Beck(1) say these processes have root caps and are roots which may form secondary haustoria on coming in contact with the same or other host roots, but Kerner(6) regards it as doubtful whether they are roots or stem-structures. In the upper part of the tubercle a bud is developed endogenously and gives rise to the flowering stem (Fig. 1, D). The union with the host is very

¹ Part of a dissertation presented for the M.Sc. degree in the National University of Ireland.

intimate, and Solms-Laubach states that the epidermis, parenchyma, soft-bast, and xylem of host and parasite meet each other very closely and that it is often difficult to distinguish the boundary between the host and parasitic tissues; Kerner states that the union is so complete that it cannot be said with certainty where the epidermis of the host ends and that of the parasite begins.

The flowering stem has a swollen base and bears a number of scale leaves. A summary of the anatomy of the plant as described by Solms-Laubach⁽¹²⁾ is as follows: The stem is chiefly composed of large cells rich in starch and tannin, surrounded by a small-celled epidermis devoid of stomata, and divided into cortex and pith by a ring of vascular bundles. In the lower swollen part of the stem there are a considerable number of vascular strands running freely in the parenchyma and arranged in a circle, which, higher up, converge and form a sinuate cylinder. Externally the bundles are composed of soft-bast, with very narrow sieve-tubes, and inwardly by dotted and reticulated vessels and wood fibres, with a few spiral vessels of very narrow lumen.

The main object in view when this work was undertaken was to study carefully the region of union between host and parasite and to endeavour to ascertain exactly how the several tissues met each other. As a necessary preliminary to a proper understanding of this, a detailed investigation of the internal anatomy of *Orobanche hederæ* was made.

METHODS

The structure of the plant was worked out from "free-hand" razor sections of fresh material or material preserved in 5 per cent. formalin, or 90 per cent. alcohol; the fresh and alcoholic materials proved much the more satisfactory. For showing the general distribution of tissues Schultz's solution and aniline sulphate were employed for staining the sections. Great difficulty was experienced in staining the phloem satisfactorily, aniline blue used as described by Pierce⁽¹⁰⁾ and Strasburger⁽¹³⁾ proving of little use. Finally, a solution of corallin in a 4 per cent. solution of sodium carbonate⁽¹³⁾ was used, and gave excellent results, both in bringing out details of the phloem, and for general tissue differentiation.

Most of the work on the early stages was done by cutting serial microtome sections of embedded material; but for the older stages free-hand sections were used entirely, and here again corallin soda proved very valuable for staining, as it differentiated the sieve-tubes

of host and parasite very well. The best thickness for cutting the paraffin sections was found to be about 10 microns, and the stain most used was safranin followed by Delafield's haematoxylin; light-green in clove oil and Flemming's triple-stain were also employed. For fixing embedded material, Bouin's picro-formol(9), and formalin-alcohol(8), gave the best results.

RESULTS

Attempts to bring about the germination of the *Orobanche* seeds in the absence of the host were always unsuccessful, but, when seeds were scattered on roots of Ivy plants growing in pots, the soil being then replaced, small *Orobanche* tubercles were found, after about two months, attached to small lateral Ivy roots. Although seeds were sown in this manner at the end of May and early in June, no aerial stems appeared above ground the same year; but the following spring several stems grew up. From this fact, and because well-developed tubercles could be found attached to Ivy roots all through the winter, it is considered probable that *Orobanche hederæ* passes at least one year entirely subterraneously before flowering. The plants first appear above ground about the beginning of May and new shoots continue to appear until the end of September.

The actual germination of the seeds was not observed and the youngest stages found were when the parasite appeared as a small nodule about one-eighth of an inch in diameter attached to small Ivy roots; in some cases remains of the decaying distal part of the embryonic filament were still discernible. At this early stage union with the xylem of the host had already been effected, so that it was not possible to study the manner in which the penetration of the host tissue was accomplished. Examination of a very large number of specimens led to the conclusion that the host root probably always dies off beyond the point of contact with the parasite, and that often the apparent continuation of the root is really a lateral branch originating near the union, as is the case in the specimen shown in Fig. 1, D.

Structure of the stem. The stem is separated into cortex and pith by a ring of vascular bundles, which vary in size, large bundles being separated from each other by one or more smaller bundles. Outwardly, the cortex is bounded by an epidermis composed of one layer of small cells, while inwardly it meets the phloem and a sheath of thick-walled lignified cells which surround the xylem portions of the bundles and intervene between them. The thin-walled cortical cells

are largest in the central region, becoming smaller outwardly and inwardly. They are more or less circular on transverse section, with intercellular spaces, and are elongated longitudinally with oblique end walls. The cells of the lignified sheath are smaller and very thick-walled where they border on the bundles and meet the cortical cells, from which they are sharply delimited. They become larger and less thick-walled towards the centre of the stem until they gradually pass into large thin-walled pith cells; sometimes the centre of the stem is hollow. The thick-walled cells are polygonal transversely, greatly elongated longitudinally, with slightly sloping end walls, and have oblique pits, which are peculiar in that the pits are elliptical

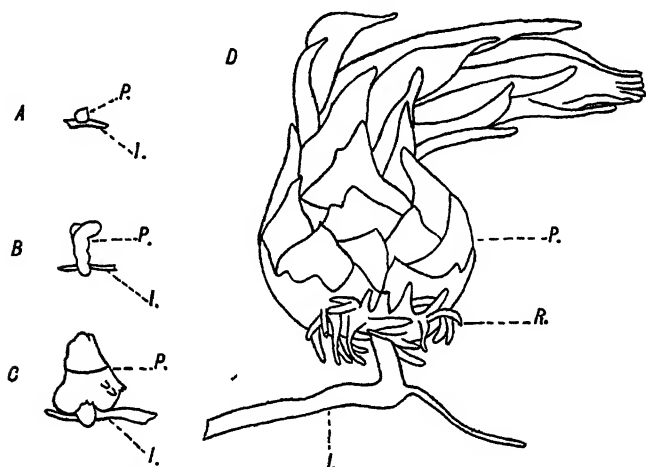


Fig. 1. Stages in the development of *Orobanche hederace*.

A, youngest stage found; B and C, slightly older stages; D, stage showing the development of roots and flowering shoot. P., parasite; I., Ivy root; R., roots of *Orobanche*.

and the inner and outer portions have their axes nearly at right angles to one another. Contrary to what Solms-Laubach (12) states, in no case were the vascular bundles seen to unite into a continuous cylinder.

The phloem is separated from the xylem of the bundle by a few rows of thin-walled cells. The phloem is chiefly composed of rather large thin-walled cells with oblique end walls and dense cytoplasm, among which are groups of very short and narrow sieve-tubes and companion-cells with dense granular contents. In old stems callus is abundant at the end walls of the sieve-tubes, and often some is also present along the lateral walls.

The xylem is formed of comparatively few vessels of small diameter arranged in groups surrounded by the lignified cells. Immediately inside the phloem are a couple of rows of thin-walled rectangular cells, then a number of narrow thick-walled cells, followed by the vessels, and finally, an area of narrow, elongated, lignified cells with pitted walls. The outermost vessels are closely reticulated, and are succeeded by less complex vessels; internally are a number of spiral vessels.

Structure of the swollen stem base. In the swollen basal part of the stem the bundles are very irregular in size and structure. There is no lignified sheath between the bundles, the epidermis is replaced by a few layers of cork cells, and there is no distinction into pith and cortex. A peculiar feature of the bundles is that the xylem is more or less central, usually consisting of a number of groups of vessels, connected by pitted cells, with a number of phloem groups arranged around them. The cortical cells are larger than those of the stem and are elongated radially. The phloem elements are very small, and the xylem is composed almost entirely of files of short tracheides, a few spiral vessels sometimes being present in addition. The groups of tracheides are separated by large, cubical cells, with rather thick, pitted and lignified walls. A few rows of small, rectangular, thin-walled cells separate the phloem and xylem.

Lower down in the region of the "tubercle" from which the roots are developed, the arrangement is extremely irregular, and the vascular bundles break up and are replaced by files of tracheides, and files of phloem elements running more or less independently of each other, and without definite orientation except that they converge irregularly towards the region of union with the host root. Tracheides are the only xylem elements present in this region.

Structure of the root. The roots are short, tapering, fleshy processes developed from the tubercle (Fig. 1, D). In no case were they observed to form secondary connections with the host, as several authors have stated to be the case. There is a central vascular region surrounded by a large-celled cortex which is bounded by a few layers of cork cells. The xylem is central and projects in a number of rays, appearing irregularly star-shaped in transverse section. A number of phloem strands lie outside the xylem, either alternating with the xylem rays or opposite to them. Radial rows of thin-walled cells separate phloem and xylem, and extend in between the xylem rays. The xylem is composed of short broad tracheides with closely reticulated walls, and the phloem resembles that found in the swollen region of the

stem. Root hairs are not developed and the roots are apparently non-functional.

The connection with the host root. The young tubercles of the parasite are found attached to small lateral roots of the Ivy, which show little sign of injury (Fig. 1, A, B, C). Later, as the tubercle enlarges, the part of the Ivy root to which the tubercle is attached becomes swollen, and it is noticeable that the part between the main root and the parasite is thicker than that beyond the point of union, which appears stunted and shrivelled (Fig. 1, C, D). In older stages, when the *Orobanche* has developed the flowering stem, the root often disappears completely beyond the junction with the parasite, where it is considerably swollen, but it may be continued as a very much thinner root beyond this point. Occasionally, it appears to be continued with little change, but in such cases the apparent continuation is probably really a lateral branch.

The outstanding features of the union are that no definite haustoria, comparable with those formed by other parasitic plants such as *Cuscuta* (10), *Melampyrum*, *Rhinanthus*, *Euphrasia* (8), are developed, the junction extending in an irregular manner over a considerable area; and that the Ivy root takes a considerable part in the development of the connection, the tissues at the point where the parasite is attached curving towards the latter, spreading out, and extending into the tissues of the parasite (Fig. 2). The tissues of host and parasite, though by no means sharply distinguished from one another, are not so closely merged together as is indicated by Kerner (6), for the meeting of the epidermis of host and parasite can always be easily recognised. There is little to differentiate the cortical cells, but those of the parasite are usually more rectangular than those of the Ivy, and have denser protoplasm and more irregular nuclei. The smaller size of the phloem elements of the parasite allows of their being distinguished from those of the host, while there is no difficulty in differentiating between the xylems, as that of the Ivy is mainly composed of large pitted vessels, while that of the *Orobanche* has only small tracheides in the region of union.

A noticeable feature of serial transverse sections of the Ivy root where it joins the *Orobanche* is the gradual disappearance of the xylem and its replacement by the parasitic cells. Before it meets the parasite the Ivy shows well-developed secondary wood, but after the junction, if it is not completely cut off, the continuation is very thin and has only a very small strand of xylem. There is very great variation in details of the manner in which the junction is effected,

but the general course followed seems to be fairly uniform. The parasite, having penetrated the epidermis and cortex, proceeds gradually to penetrate the xylem region, meanwhile sending off lateral processes which extend through the cortex and phloem of the host, encompassing the xylem, and finally meeting at the opposite side. At the same time, the outer, less strongly lignified xylem elements are encroached upon by the parasite. The central small-celled and strongly thickened xylem elements are apparently very

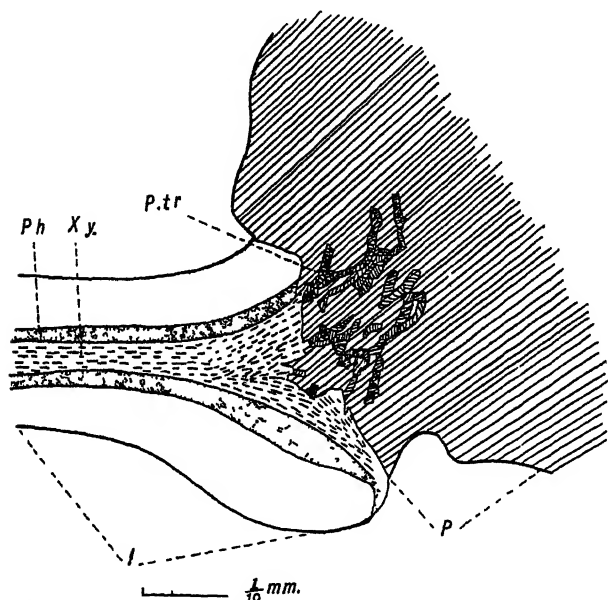


Fig. 2. Median longitudinal section through the region of junction of host and parasite. The *Orobanchae* tissue is shaded *I.*, Ivy root; *Ph.*, phloem of Ivy; *Xy.*, xylem of Ivy; *P.tr.*, files of reticulated tracheides of the parasite meeting the xylem of the host.

resistant to the enzymes secreted by the invading cells, and frequently persist as a small strand entirely surrounded by parasitic tissue. While the encompassing of the xylem is in progress, the parasite also extends longitudinally into the host root, growing beneath the cork layer of the root, which is usually raised and curved upwards, and sending blunt processes into the cortical tissue and the xylem.

The behaviour of the Ivy root is best seen in longitudinal sections through the junction with the parasite (Fig. 2). The penetration of

the parasite seems to stimulate the cambium of the part of the root immediately in front of it to increased activity. The cells thus developed become curved and elongated in a radial direction on the side to which the parasite is attached, forming an area of tissue, the cells of which are elongated and directed towards the parasite. Certain of these cells soon become lignified and thick-walled, developing into pitted tracheides or tracheae. The longitudinal extension of the parasite has meanwhile been proceeding and the lignified cells are met at varying heights by the advancing cells of the parasite. These invading cells apply themselves closely to the Ivy vessels on

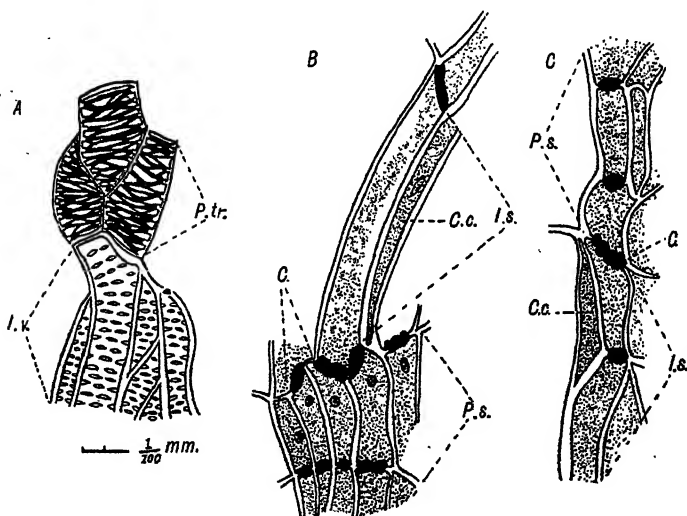


Fig. 3. *A*, group of tracheides of the parasite meeting a vessel of the Ivy root. *B*, two parasitic sieve-tubes meeting one host sieve-tube. *C*, end to end union of a parasitic and a host sieve-tube. *I.v.*, vessels of Ivy; *P.tr.*, tracheides of the parasite; *I.s.*, sieve-tubes of Ivy; *P.s.*, sieve-tubes of parasite; *C.c.*, companion cells; *C.*, callus.

coming in contact with them, and force their way in between them in such a manner that the newly formed Ivy vessels form a series of irregular projections into the parasite. After this stage has been reached, the arrangement becomes extremely complicated by the increased growth of the root and the extension into it of processes of the *Orobanche*. At the time of flowering the junction extends over a considerable area; and, since both host and parasite send extensions into each other, the boundary line between them pursues an extremely irregular course.

The meeting of the epidermal and cortical tissues presents no features of special interest.

Narrow strands of phloem in the parasite converge towards the point of entry into the host, and meet the phloem of the Ivy root. The sieve-tubes of host and parasite come into close contact and, as far as was observed, the union is always end to end (Fig. 3, *B, C*). In old plants, where callus was well developed, the meeting of the sieve-tubes could be easily observed, and in such cases the callus was much more abundant on the side of the sieve-plate on which the host cell was situated, than on the side occupied by the parasitic sieve-tubes. Often a couple of the small sieve-tubes of the parasite are connected with the end of one of the large sieve-tubes of the Ivy (Fig. 3, *B*).

The parasitic cells which apply themselves to the vessels of the Ivy, enlarge and develop reticulate thickenings, forming small tracheides (Fig. 3, *A*). The tracheides are developed in files continuous with those running in the body of the tubercle (Fig. 2). Each of the host vessels which take part in the union has, as a rule, a group of tracheides clustered around its apex, these being the termination of a file of one or more rows of tracheides running in the body of the tubercle (Fig. 3, *A*). In some cases, the thin areas between the reticulations of the tracheides were seen to correspond with the thin areas of the dotted vessels with which they were in contact, but this was not always the case. The tracheides are frequently connected with the lateral walls of the host vessels, being directed either parallel with them or at right angles to them.

SUMMARY

1. Seeds of *Orobancha hederæ* do not germinate in the absence of the host plant.
2. The aerial stem is distinguished into a large-celled cortex and pith by a sinuous ring of vascular bundles of varying sizes, the xylem regions of which are surrounded and connected together by a sheath of thick-walled lignified cells. The xylem region comprises reticulated and spiral vessels and elongated thick-walled cells.
3. The phloem and the vessels of adjacent vascular bundles do not unite with one another in any region of the stem.
4. The arrangement of the vascular tissue is very irregular in the swollen stem base, and the xylem is formed almost entirely of tracheides and is surrounded by a number of phloem strands.

Tracheides are the only xylem elements present in the lower region of the swollen base.

5. The roots are short and composed of large, starch-containing cells surrounding the central vascular bundle. The xylem is central with projecting rays and contains only tracheides. The phloem is arranged in strands alternating with, or lying opposite to, the xylem rays.

6. No definite haustoria are formed where the parasite enters the host, but the union extends in an irregular manner over a considerable area.

7. An important part in the development of the connecting tissue between host and parasite is taken by the Ivy.

8. The cortical cells of host and parasite come into close contact.

9. Sieve-tubes of host and parasite meet end to end.

10. The xylem of the host is connected with that of the parasite by files of short reticulated tracheides.

11. It is regarded as probable that, normally, the Ivy root always dies off beyond the attachment of the parasite.

In conclusion the author wishes to express his thanks to Professor H. A. Cummins, University College, Cork, at whose suggestion the work was undertaken, for much help throughout; and to Mr F. T. Brooks, University of Cambridge, for advice in revising the manuscript.

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A NOTE ON THE RELATION OF RATE OF GROWTH TO STRUCTURE IN PLANTS

By LIONEL S. PENROSE

(With 4 figures in the text)

“THE form of an organism is determined by its rate of growth in various directions.” This statement is made by D’A. W. Thompson in *Growth and Form*, and the writer goes on to say that the ratio between rates of growth in various directions may sometimes be of a simple kind, as when growth results in the definable outline of a shell or the smooth curve of a leaf¹. It is the purpose of this paper to amplify this idea in regard to one or two special cases of plant growth, by showing how certain common forms can be referred (a) to relations between the rates of growth of their parts, and (b) to the rate of growth of the whole plant at different periods.

Let us consider a simple case of growth in length, where the successive production of new parts takes place by the division of an apical cell or group of cells, and where the parts are formed one after another along a straight axis. Let us also suppose that each new part possesses growth properties in certain respects similar to those possessed by the apical group of cells. A method of growth of this kind leads to the formation of repeating structures, such as are to be found in the forms of the higher plants, where each part is similar in shape to the whole. Obvious examples are to be found in the fronds of the Male Fern and the leaves of Fool’s Parsley. In each of these instances the ability of the part to repeat the structure of the whole is expressed in three stages, the last being incomplete. More commonly the repetition in shape does not reach further than one stage, in that the parts are not themselves similar in shape to the whole, but only to one another. At the same time the growth of each part may repeat the growth of the whole, by keeping the ratio of its size to that of the whole plant constant. The dimensions of successive members form in this case a geometrical progression provided that the system of growth is uniform (Fig. 1). And where the production of each new part takes place at a constant angle from the last around the axis of growth, the same geometrical progression appears in cross section as a logarithmic spiral².

¹ p. 53.

² Cf. A. H. Church, *On the Interpretation of the Phenomenon of Phyllotaxis*. 1920.

If the uniform growth of the plant is taken to mean that the initiation of growth in each new part takes place at equal intervals of time, it is possible to ascertain the rates of growth of the parts of a plant by measuring a series of these parts at different stages of development¹. In the simplest case, where successive parts are arranged in geometrical progression, either directly along an axis, or in the form of a logarithmic spiral, a compound interest rate of growth can be inferred for the whole and for each individual part. But the exact spirals which are formed by growth at the top of the axis of a plant are seldom continued very far down the stem. Although

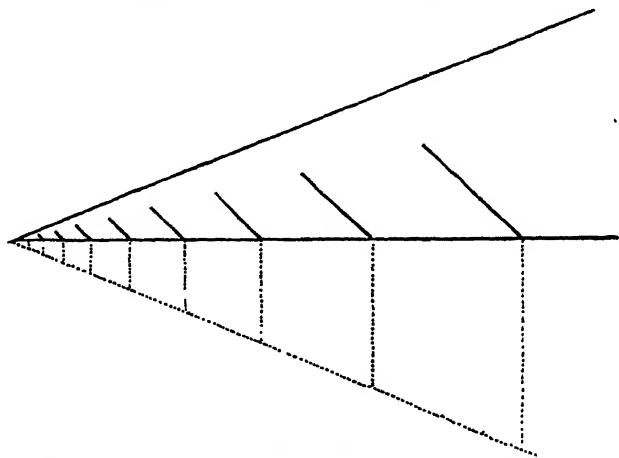


Fig. 1. Diagram showing the outline of a growing structure where each similar part formed at the apex, as well as the whole, increases according to the compound interest law. The outline is a straight line.

the system of growth may remain the same, after a certain point a retardation is observable, and the parts of the plant which originally formed a geometrical series as regards their dimensions tend to approach a uniform limit of size.

By taking measurements of the successive leaves and their corresponding internodes along the axis of a growing plant and by plotting these lengths at equal intervals (which, by hypothesis, represent time intervals), curves showing the rate of growth of these parts are obtained (Fig. 2). Curves deduced in this way from various plants show a considerable uniformity, and agree with observations made on the rate of growth of parts of plants by other methods².

¹ This method is suggested by J. Sachs, *Lectures on Plant Physiology*, p. 545. Oxford. 1888.

² J. Sachs, *op. cit.* p. 540.

There is in fact in the rate of growth of each part, as in the whole plant, an initial phase of increase, followed, after a period of even growth, by a similar decrease.

The suggestion has often been made that these changes in the rate of growth of plants correspond to the rate of progress of an auto-catalytic chemical reaction¹. That is to say, the compound interest increase of the substance of a plant is subject to a sliding scale capital levy. Though it has been pointed out that where dry-weight is concerned this law holds only very approximately², yet the results which would be manifest in the form of a plant if linear growth or expansion of each part in a given direction were subject to this law are worthy of consideration.

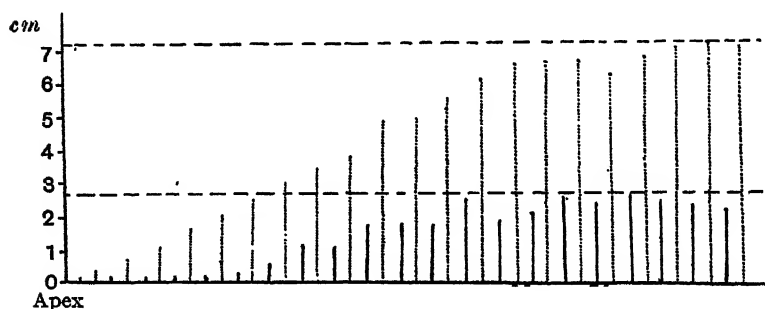


Fig. 2. Graph showing the lengths of successive leaves and internodes in a specimen shoot of Almond. Lengths of leaves, dotted lines, of internodes, continuous lines.

If each similar part along a growing axis increases in length according to the law $t = \log \frac{x}{A-x}$ (where t measures an interval of time, x the length at the end of the interval and A the limit which x approaches)³, then the total length of the axis, when the parts form a continuous line, is found by integrating x with respect to t . The resulting formula for the length (L) after t repetitions is of the form $L = A \log (1 + e^t)$. In order to find the curve of outline of the plant where transverse growth is proportional to growth along the axis in each section, and where the successive parts are very numerous and fused as in the frond of the Hart's Tongue fern, a value proportional to x is plotted against L . This ultimate relation

¹ T. B. Robertson, *The Chemical Basis of Growth and Senescence*, p. 3. 1923.

² C. West, G. E. Briggs and F. Kidd, "Methods and Significant Relations in the Quantitative Analysis of Plant Growth." *New Phyt.* 19, p. 202.

³ T. B. Robertson, *op. cit.* p. 6.

between the transverse and longitudinal growth is found, on eliminating t from the two above expressions, to be $x = A (1 - e^{-L/A})$. This gives the curve of outline of a plant structure where apical growth is concerned, and, by comparing it with the outline of a given plant structure, the rate of growth of the parts can be studied (Fig. 3).

The assumption hitherto involved that all similar members tend to reach the same limiting size needs much attention. It does not seem to be generally true except in the middle regions of the axis of growth. Not only the lowest leaves but also those which unfold late in the season or those which immediately precede the floral structure are smaller than the maximum size in numerous flowering

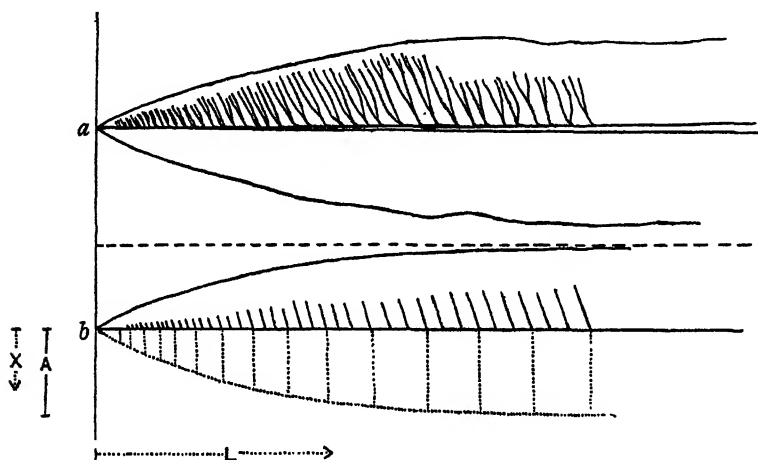


Fig. 3. (a) The apex of a frond of Hart's Tongue fern drawn to scale and compared with (b) a theoretical construction in which growth in each similar part, in a continuous structure with apical growth, is retarded according to the autocatalytic law. The outline curve is logarithmic.

plants. The same applies to the final lengths of the various internodes, and this is well illustrated by the distribution of the leaf scars upon the part of a twig of a deciduous tree which represents one year's growth. It would thus appear that there is a gradient of potency as regards the extent of growth in each similar part which first increases and then decreases as the axis of growth is ascended.

The distribution of the main veins along the midrib of an ovate leaf is very often comparable to the arrangement of branches or leaves upon the twig of the plant, in that the distances between such consecutive veins increases and then decreases as the midrib is followed up towards the apex. The distances involved here are easy

to measure and to average, and (from a great number of measurements of leaves, including those of the Horse Chestnut, Walnut, Privet and Ash) the average distribution of the veins in the middle leaflet of *Ptelea trifoliata* (50 specimens, both sides of the leaf measured each time) may be taken as an example.

Length of the leaflet—1 unit.

Average distances of the origins of the main veins from the base, which was taken to be the point where the other two leaves arise: .070, .106, .151, .207, .276, .345, .424, .502, .587, .663, .733, .794, .842, .887 and .915.

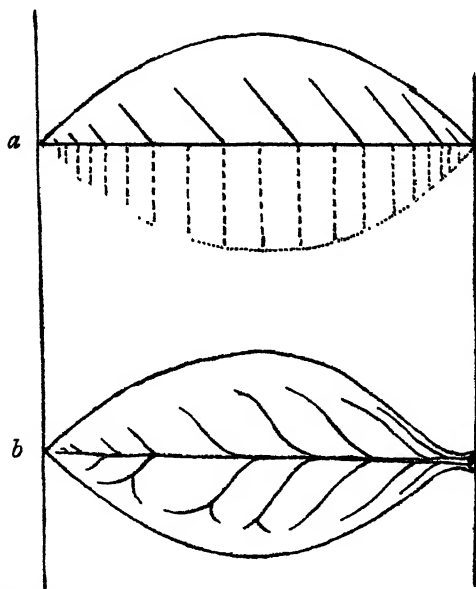


Fig. 4. (a) Diagram showing the outline of the structure formed if each similar part, as well as the whole, grows with autocatalytic retardation, the limiting size of each part being determined by the rate of growth of the apical growing point when it originated.

(b) A typical Privet leaf drawn to scale (length 5.9 cm) for comparison with the construction.

To account for this series, and others of the same character, it can be supposed that the limiting size to which the similar parts of a plant structure attain (the parts being in this case internodes along a straight axis) is determined by the rate of growth of the growing area when they are originated, and that this rate increases and decreases according to the law previously stated, which corresponds to the autocatalytic reaction. Under these circumstances the struc-

ture once formed would have the same proportions at all subsequent stages of growth and would simply appear to swell out. Furthermore, if transverse growth in each similar part were directly proportional to longitudinal growth and if the parts were all fused together, the outline of the structure so produced would have the form of a parabola (Fig. 4). And many ovate leaves closely approximate to this theoretical criterion.

A series of numbers calculated upon this assumption for the distances of the origins of side shoots along the axis from the base may be compared with the above observed figures, *e.g.* .081, .111, .150, .200, .261, .333, .414, .500, .586, .667, .739, .800, .850, .889 and .919. The formula used is, $n = 2 \log_2 \left(\frac{y}{1-y} \right)$, where y is the distance of the origin of the side shoot or vein from the base, and n is the number of such shoots or veins.

Only some of the simplest possibilities regarding the relation between rate of growth and structure in plants have been discussed in this paper, but the assumptions which have had to be made are those which appear to be in harmony with what other methods of investigation in related spheres have warranted. In particular the work of J. Sachs, A. H. Church and T. B. Robertson has been consulted.

ON *TRACHELOMONAS HISPIDA* (PERTY) STEIN AND ITS VARIETIES

By B. W. SKVORTZOW
Harbin, Manchuria, China

(With 17 figures in the text)

IN this small contribution I give a short description of all the known varieties of *Trachelomonas hispida* (Perty) Stein.

This alga belongs to the green Flagellata of family Eugleninae. In 1838 this alga was described by Chr. G. Ehrenberg as *Chaetoglena volvocina* Ehrenberg, in 1852 by M. Perty as *Chonemonas hispida* Perty and *Ch. Schrankii* Perty, and only in 1878 it was assigned to the genera *Trachelomonas* Ehrenberg by F. Stein and named *Tr. hispida* (Perty) Stein.

From 1857 to 1922 in a long series of notes and short papers by Maskell, Schröder, Lemmermann, Swirenko and myself there are to be found descriptions of forms related to *Tr. hispida*. The varieties var. *cylindrica* Klebs, var. *margaritifera* (H. Kufferath) Conrad, var. *granulo-spina* Drezepolski, var. *irregularis* Drezepolski, var. *Niezabitowskii* Drezepolski, var. *setosa* Drezepolski and var. *verrucosa* Drezepolski seem to me to belong to other species and are therefore not included in my list. Some new varieties have recently been found in Manchuria, and a description of these is given below.

1. *Trachelomonas hispida* (Perty) Stein. Fig. 1.

Stein(21), Fig. 21, 24-33; Klebs(7), p. 319; Dangeard(2), pp. 134-135, Fig. 41; *Chaetoglana volvocina* Ehrenberg(3), p. 352, Taf. XXII, Fig. 12; *Chonemonas hispida* (Perty)(23), p. 166, Taf. X, Fig. 11-12; *Ch. Schrankii* Perty; Lemmermann(4), p. 526, Fig. 14-15; Pascher u. Lemm.(10), pp. 149-150; Swirenko(12), p. 637; (11), p. 21; Skvortzow(14), p. 69; (15), p. 13; (16), pp. 19-20; (19), p. 192; Wislouch(22), p. 262, Taf. V, Fig. 3; var. *minima* H. Kufferath(8), p. 258, Fig. 10; W. Conrad(1), p. 206; *J. superba* Swir. in Drezepolski(24), p. 222, Fig. 44.

Shell light or dark brown, oval or elliptic, covered with long sharp pointed spines, 19-46 μ in length, 12-35 μ in breadth. The aperture for the flagella is wide, often with a straight tube-like neck. Flagella 2-3 times longer than the shell. Chromatophores 8-10. Stigma distinct.

Geogr. distribution: Europe, Asia, America, in marshes and in plankton.

2. Var. *subarmata* Schröder. Fig. 2.

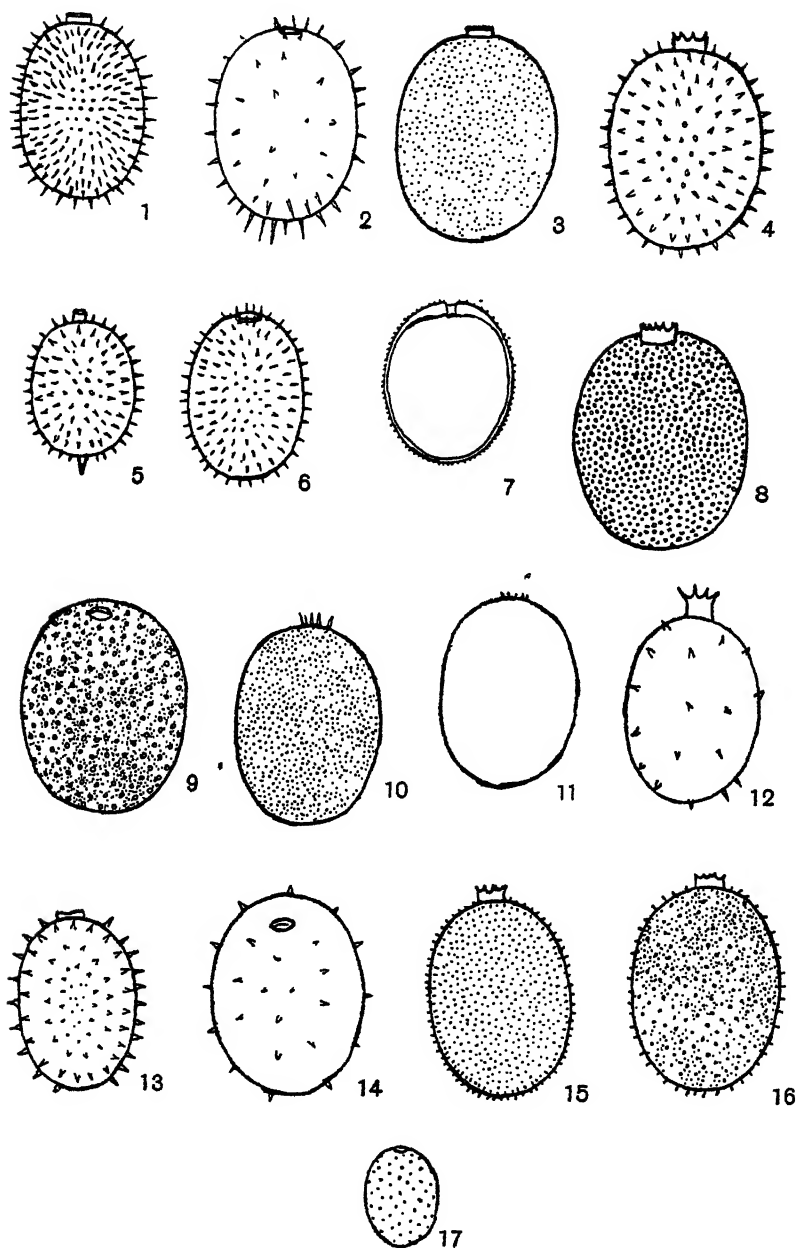
Schröder(20), v. Teil, p. 49, Taf. XI, Fig. 7; Lemmermann(4), p. 527; Pascher u. Lemmermann(10), p. 150; Swirenko(12), p. 637; Skvortzow(14), p. 69; W. Conrad(1), p. 207; Drezepolski(24), p. 216, Fig. 50.

Shell brown, covered with sharp pointed spines. On the ends of the shell the spines are longer than those of the middle parts. Shell 31-47.5 μ in length, 23-35 μ in breadth; in other respects similar to the typical form.

Geogr. distribution: Europe, Russia, North Manchuria (China).

3. Var. *punctata* Lemmermann. Fig. 3.

Lemmermann(5), Bd. 18, p. 165; (4) p. 527; Pascher u. Lemmermann(10), p. 150; Swirenko(11), p. 21; (12), p. 637; Skvortzow(14), p. 69; (19), p. 192; W. Conrad(1), p. 208; *J. lukoviensis* Drezepolski(24), p. 217, Fig. 33.



Figs. 1-17. Varieties of *Trachelomonas hispida* (Perty) Stein.

Shell brown, dotted, without spines, 23–35 μ in length, 18–25 μ in breadth. Chromatophores numerous. Stigma distinct.

Geogr. distribution: Europe, Russia, South China, North Manchuria.

4. Var. *crenulatocollis* (Maskell) Lemmermann. Fig. 4.

Tr. crenulatocollis Maskell (9), p. 52, Taf. III, Fig. 3; Stein (21), Taf. XXII, Fig. 20; Lemmermann (4), p. 526; Pascher u. Lemmermann (10), p. 150; Swirenko (12), p. 637; Skvortzow (14), p. 69; W. Conrad (1), p. 207; *J. hispida* var. *coronata* Lemm. in Drezepolski (24), p. 215, Fig. 51.

Shell oval, brown, 26–37 μ in length, 15–26 μ in breadth, covered with spines. The tube-like neck is serrated, 3 μ in length, 5 μ in breadth, and in the upper part somewhat enlarged. Flagella 2, longer than the shell. Chromatophores 10.

Geogr. distribution: Europe, Russia, New Zealand, North Manchuria.

5. Var. *caudata* Lemmermann. Fig. 5.

Lemmermann (4), p. 526; Stein (21), Taf. XXII, Fig. 22.

Shell brown, covered with spines. On the lower end the shell has a large pointed colourless spine; in other respects similar to the typical form.

Geogr. distribution: Germany.

6. Var. *coronata* Lemmermann. Fig. 6.

Pascher u. Lemmermann (10), p. 150; *Tr. robusta* Swirenko (12), p. 636, Taf. XIX, Fig. 17; Skvortzow (14), p. 69; W. Conrad (1), p. 207; Drezepolski (24), p. 215, Fig. 47.

Shell oval, 30–37 μ in length, 22.5–26 μ in breadth, covered with sharp pointed spines. The aperture for the flagella surrounded with large pointed spines; in other respects similar to the typical form.

Geogr. distribution: Germany, Polen, Russia, North Manchuria.

7. Var. *incrassata* Swirenko. Fig. 7.

Swirenko (11), p. 28, Taf. I, Fig. 34.

Shell oval, brown, covered with minute spines 35 μ in length, 26 μ in breadth. The anterior part of the shell is thickened; in other respects similar to the typical form.

Geogr. distribution: Russia.

8. Var. *macropunctata* Skvortzow. Fig. 8.

Skvortzow (18), p. 51, Pl. I, Fig. 24.

Trachelomonas hispida (Perty) Stein and its varieties 303

Shell oval, brown, 32μ in length, 25μ in breadth, covered with circular pits. The tube-like neck is serrated and 5.8μ in length.

Geogr. distribution: Up to the present found only near Charbin.

9. Var. *bipunctata* Skvortzow. Fig. 9.

Skvortzow (18), p. 51, Pl. I, Fig. 11.

Shell brown, oval. The surface is dotted and covered with circular pits. The length is 26μ , the breadth 21μ . Aperture for flagella 3.7μ in breadth. Chromatophores numerous; in other respects similar to the typical form.

Geogr. distribution: Only in North Manchuria, in marshes at Harbin.

10. Var. *punctulosa* var. nov. Fig. 10.

Shell oval, with minute dots, 25μ in length, 18μ in breadth. Cilia aperture is surrounded with spines; in other respects similar to the typical form.

Geogr. distribution: Up to the present found only near Harbin, North Manchuria.

11. Var. *papillata* var. nov. Fig. 11.

Tr. acanthostoma var. *rossica* (Swil.) Skvortzow (14), p. 66, Taf. V, Fig. 10.

Shell dark brown, smooth, without spines, $34-40\mu$ in length and $28-30\mu$ in breadth. The aperture for the flagella is surrounded with spines; in other respects similar to the typical form.

Geogr. distribution: Only in North Manchuria.

12. Var. *simplex* var. nov. Fig. 12.

Shell oval, brown, 37μ in length, 23.5μ in breadth, covered with few spines. The tube-like neck is serrated, 5.7μ in length and $3.7-4\mu$ in breadth. Chromatophores numerous. Stigma distinct.

Geogr. distribution: Near Harbin (North Manchuria), in marshes.

13. Var. *charkowiensis* (Swirenko) nov. Fig. 13.

Tr. charkowiensis Swirenko (13), p. 21, Taf. XI, Fig. 5; (12), p. 641, Taf. XIX, Fig. 5.

Shell oval, light brown, 46μ in length, 35μ in breadth. The tube-like neck 6μ in length, 2μ in breadth. The aperture for the flagella serrated and enlarged. Shell is covered with colourless spines 4μ in length. The lower part of the shell is spineless.

Geogr. distribution: Russia, in marshes in the Charkow Government.

14. Var. *hyalina* var. nov. Fig. 14.

Tr. charkowiensis Swirenko in Skvortzow (14), p. 68, Taf. IV, Fig. 14. Shell colourless, without neck or spines; in other respects similar to the typical form.

Geogr. distribution: In marshes near Harbin, North Manchuria.

15. Var. *spinulosa* var. nov. Fig. 15.

Shell brown, 20μ in length, 15μ in breadth, covered with minute spines. The neck is serrated; in other respects similar to the typical form.

Geogr. distribution: North Manchuria.

16. Var. *spinopunctulosa* var. nov. Fig. 16.

Shell brown, 25μ in length, 18μ in breadth, covered with minute dots and little spines. The neck is serrated; in other respects similar to the typical form.

Geogr. distribution: North Manchuria.

17. Var. *volicensis* Drezepolski. Fig. 17.

Drezepolski (24), p. 216, Fig. 35.

Shell dotted, $17-22\mu$ in length, $11.5-15\mu$ in breadth. Dots large and rare. Aperture for the flagella without a tube-like neck.

Geogr. distribution: Poland.

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HARBIN,
June, 1925.

A NOTE ON CALAMOSTACHYS TUBERCULATA STBG.

By ISABEL M. P. BROWNE

It has long been known that the fructifications called *Calamostachys tuberculata* Stbg. were borne in whorls of twelve to sixteen on main branches, or possibly on main stems, of the type of *Annularia stellata* Schl. (= *A. longifolia* Bgnt.). They were heterosporous cones, 7-15 cm. long, and consisted of whorls of 16 to 32 bracts, midway between which were whorls of 8 to 16 sporangiophores¹.

So much is generally accepted. But when we come to consider the distribution of bracts and sporangiophores on the axis we find that the records are obscure and sometimes conflicting. The species is one of which we possess not only impressions but petrifications, our knowledge of the latter being due entirely to Renault, who discovered that the cone was heterosporous. This botanist found considerable portions of decorticated and silicified cone-axes with some of the bracts and sporangiophores still *in situ*. Besides giving figures of other petrified portions of the cone Renault repeatedly figured

¹ Jongmans, 1911, pp. 293-295.

these decorticated axes¹. In all these figures it can be seen that the bundles are vertically continuous from one internode to the next and that the sporangiophores are inserted opposite to them and therefore arranged in superposed whorls. Weiss also noted the constant continuity of the axial ribs of the cone, which, of course, correspond to the bundles in the decorticated specimens². Nevertheless, the only statement made by Renault as to the course of the bundles of the cone and their position with reference to the sporangiophores is to the effect that the bundles alternate and that the sporangiophores are equal in number to them and inserted on them³. If these statements were correct they would, of course, involve the alternation of the sporangiophores of successive whorls. This has recently been pointed out by Dr Hirmer⁴, who neither makes himself responsible for the accuracy of Renault's statement nor mentions the conflict between it and the figures, though he criticises the drawing of some of the latter, pointing out that they do not show the positions of the appendages with any exactness. Renault's figures, it may be noted, seem to be in disagreement and his statement in agreement with Grand' Eury's work; for though the latter does not describe the course of the bundles he gives two figures of impressions of this cone in which the ribs of the axis alternate regularly at the levels of insertion of the bracts⁵. In both figures, though more clearly in the smaller one, the sporangiophores of successive whorls appear to alternate with one another and their points of insertion seem to be between the bundles. Grand' Eury's figures, however, give the impression of being restorations.

Though figures of impressions of cones of *C. tuberculata* are numerous⁶ the large number of the bracts in a whorl and the crowding of the appendages make it very difficult to determine from them whether the bracts and sporangiophores were superposed to or alternated with the members of similar whorls, though some of them show fairly well the continuity of the ribs of the axis. One of Weiss' figures does, indeed, suggest superposition of the sporangiophores⁷, but it is not entirely satisfactory and we know that in the specimen figured sporangia and sporangiophores were displaced and deformed during fossilisation⁸.

¹ Renault, 1873, Plate 20, figs. 7 and 8; 1878, Plate 1, fig. 1; 1882, Plate 21, fig. 2; 1893, Plate XXVIII, fig. 4.

² Weiss, 1876, p. 20.

³ Renault, 1890, pp. 403-405.

⁴ Hirmer, 1925, p. 243.

⁵ Grand' Eury, 1877, Plate VI, figs. 4 and 4'.

⁶ For the synonymy and iconography of this cone see Jongmans, 1915, p. 493.

⁷ Weiss, *Atlas*, 1876, Plate III, fig. 4.

⁸ Jongmans, 1911, pp. 283-284.

In view of this conflict of evidence I was particularly grateful to M. Costantin, Director of the Museum of Natural History in Paris, for allowing me to examine Renault's original specimens. These show quite clearly that as regards the course of the bundles or ribs Renault's figures are correctly drawn and that his statement that the bundles alternate is wrong: the bundles are clearly continuous and the sporangiophores inserted opposite to them and therefore in superposed whorls. I was also able to examine an ill-preserved transverse section of the axis passing through the sporangiophores and in it, too, the bundles were opposite to the sporangiophores, though in two cases the opposition was not quite accurate¹.

In one respect, however, Renault's figures of decorticated axes are inaccurate. He had begun² by describing the bracts as being equal in number to the sporangiophores, but soon corrected this mistake and in his later publications gave them as twice as numerous as the latter³. Nevertheless, he continued in his figures to draw the bracts and their scars as though they were about equal in number to the sporangiophores. This is especially clear in his largest-scale figure⁴, in which the bracts or their scars are drawn as lying alternately with and between the bundles. In the original specimens, however, the bracts and bract-scars are, as might be expected from the impressions, twice or approximately twice as numerous as the bundles and sporangiophores. They seem to lie indiscriminately and slightly irregularly on and between the ribs or bundles. They are nearly contiguous and their crowding and the poor preservation of the axes make it very difficult to be sure whether the bracts of successive whorls alternated with or were superposed to one another⁵. On the whole, the arrangement seemed to be a slightly irregular alternation, the irregularity being probably due to a change in the number of bracts, since it is known that in several species of *Calamostachys* these vary in number in different whorls of the same cone. A study of the iconography of

¹ This section is very similar to that represented in Renault's Fig. 9 of Plate 21 of 1873 and in his Fig. 5 of Plate 2 of 1878, but there is no sign of the very small irregularly placed lacunae seen in those figures.

² Renault, 1873.

³ As Dr Hirmer (1925, p. 243) points out the statement on p. 130 of Vol. 2 of the *Cours de Botanique fossile* (Renault, 1882) that the sporangiophores were twice as numerous as the bracts is due to a misprint, since elsewhere in the same account the former are correctly stated to be half as numerous as the latter.

⁴ Renault, 1878, Plate 1, fig. 1.

⁵ In Grand' Eury's figures, already alluded to, the bract-scars are correctly drawn as so crowded that it is not possible to be sure whether they alternated with or were superposed to one another.

the impressions, though also inconclusive, tends to support this view¹.

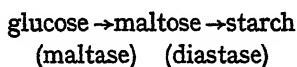
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THE RÔLE OF CANE SUGAR IN THE PLANT

By ROBERT E. CHAPMAN

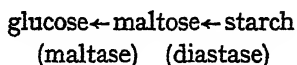
IN the recent interesting paper by Parkin⁽²⁾ on the first sugar of photosynthesis, the writer appears to have missed some relevant facts. He states (p. 59, para. 1) that with glucose as the initial and starch as the final carbohydrate formed in the cell, the disaccharide present should be maltose, instead of cane sugar. Maltose is invariably absent from the green leaf which contains starch, as Davis⁽¹⁾ and his co-workers at Rothamsted showed in their very extensive researches on the carbohydrates of the leaf; but the enzyme maltase is as invariably present in starch-forming plants. The leaf-cell then contains the system



The absence from the cell of maltose, although it must be formed in the production of starch from glucose, is easily explained by the assumption that the Reaction Velocity of the first part of the reaction is less than that of the latter, so that any maltose, as soon

¹ Weiss' fig. 4 of Plate III of the publication of 1876, a figure to which allusion has already been made, however, rather suggests inaccurate superposition of the bracts.

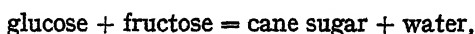
as it is formed, is at once changed to starch. On the other hand, in the reverse reaction



the reaction catalysed by diastase has a lower Reaction Velocity than that catalysed by maltase, and again no maltose would be found in the cell. (That is, in the reaction catalysed by maltase the equilibrium point is near glucose, and in that catalysed by diastase, the equilibrium point is near to starch.)

The fact that in experiments of feeding detached leaves with sugar solution cane sugar is a better starch-former than invert sugar or maltose, cannot, as Parkin suggests (p. 59), be taken as evidence that cane sugar may be directly synthesised into starch, or that "nascent hexoses" are more readily transformed into starch than when in other more stable conditions. The results may equally well be interpreted as showing that the plant cell is more permeable to cane sugar than to other sugars. If Parkin can show that, as the result of feeding experiments, the internal concentration of, say, invert sugar is the same as the internal concentration of cane sugar, then he will have strong support for his theory, but until then, it is "non-proven."

The formation of cane sugar in meristematic tissues would seem to be extremely probable, if the cells are permeable to hexose sugars. The reaction involved is



and in a condition of equilibrium we have

$$\frac{(\text{conc. cane sugar})(\text{conc. water})}{(\text{conc. glucose})(\text{conc. fructose})} = K \text{ (a constant).}$$

To obtain the formation of cane sugar, the best way is to diminish the concentration of water. But in the non-vacuolated, protein-synthesising cells of a meristem, this is exactly what occurs—the water content is reduced to a minimum. Hence if hexoses and invertase are present, the formation of cane sugar seems inevitable.

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REVIEWS

The Phylogenetic Method in Taxonomy. HALL, H. M., and CLEMENTS, F. E. *Carnegie Inst. Publ.* No. 326. July 1923. 346 pages, 58 Plates.

The high standard of work attempted in this book may be gauged from the following passage in the introduction:

To be both comprehensive and thorough, taxonomy must draw its materials from all other fields, just as it must serve them in turn. While it leans most heavily upon morphology, it cannot afford to neglect histology and physiology, and it must learn to go hand in hand with ecology and genetics in the future. Indeed, if it is to reflect evolution as accurately as it should, it must regard physiological adjustment as the basic process, and morphological and histological adaptations as the measurable results. This means that the taxonomist of the future will think in terms of evolutionary processes, and will learn to treat his morphological criteria as dynamic rather than static.

When capacious and efficient minds aim at lofty ideals they are obliged to reach something worth attaining; so it is not surprising, when Hall and Clements write monographs with such a wide conception of Taxonomy, that they should produce a book abounding in interesting matter. The body of this work consists of monographs of the North American species of *Artemisia*, *Chrysothamnus* and *Atriplex*. When reviewing monographs, it may seem trite to say that emphasis has been placed on Phylogeny as a basis of Classification, because all present-day systematists suppose that Phylogeny is the sole basis of proper Classification. But it is obvious that the authors of these monographs have been conspicuously faithful to this ideal. These are monographs in which systematists may with enjoyment lose their way in wide phylogenetic rambles, and in which the reviewer may with equal ease lose his way. It is interesting to note, in view of the enormous number of characters which have been assimilated and digested, that the authors have been led to the acceptance of a very broad species concept, and to the treatment of the natural units within each species as sub-species. These sub-species bear trinomial designations. Thus the species of *Atriplex* of the section *Teuthopsis* are regarded as sub-species of *Atriplex patula*, and called *Atriplex patula hastata*, *A. patula glabriuscula*, *A. patula typica*, *A. patula litoralis*, etc. It will be seen that these sub-species correspond with the species used by Moss and Willmott in the Cambridge British Flora. Smaller units than the sub-species are placed under the heading "Minor Variations and Synonyms," which follows the description of all the sub-species into which a species is divided.

These minor variations and synonyms are arranged alphabetically, and bear numbers which are referred to, when necessary, after the description of the sub-species with which they are associated.

Trinomial designations for sub-species cannot be expected to become current, and we think it would have been better to have called these plants *A. hastata*, *A. glabriuscula*, etc., and to have expressed their consanguinity by means of a heading. If the word "Section" has too broad a meaning for use in such a heading, then some word equivalent to the German "Gesammtart" might be found.

Throughout the monographs the result of work in the field rather than that done in the herbarium has been applied to problems of differential diagnosis and classification. Of special value is the statistical method of studying characters. Various characters have been examined in thousands of plants, and the result of the examinations embodied in tables. Hard work at gathering facts and sanity in making deductions from them are the key-notes of the work.

The phylogenetic charts are novel and interesting. They are in the form of genealogical trees, in which the branches are labelled with the diagnostic characters of the species or groups of species which they bear, so that the charts can be used as keys for determining species.

The accounts of the ecology and uses of the plants are very praiseworthy. It is refreshing, after examining the detailed account, which is followed by extensive statistical tables, of the 20 sub-species and the 83 minor variations and synonyms of *Chrysothamnus nauseosus*, to learn that "Field surveys indicate that perhaps 300,000,000 pounds of rubber of good grade are present in the wild shrub of this species in the Western United States."

All the accepted species and many of the sub-species and minor forms are illustrated by half-tone plates, which are botanically impeccable, but lack beauty. It is a pity that modern pictures of plants cannot give pleasure as well as profit. Many of the plates include sketches illustrating the habit of the plant. These rough sketches of the living plant are of great importance. Systematists, especially those who work much in herbaria, are apt to forget that the habit of a plant is often as important as any other character. We would do well to remember that the British Elms and some of the Poplars can be distinguished more readily by photographs of living specimens than by drawings of their innovations. The systematist who classifies trees too often confines his studies almost entirely to their twigs. H. G.-C.

The London Catalogue of British Plants. 11th Edition. 1925.
Price 10d. London, George Bell and Sons.

All interested in the British Flora will welcome the 11th edition of the *London Catalogue of British Plants*. The 10th edition was published in 1908, and during the interval of 17 years between the two editions many new plants have been found in the British Isles. Critical study too has added to the bulk of the British Flora; thus, the number of *Hieracia* has increased from 133 to 248. Though almost every page of the new edition reveals some change, yet the catalogue remains in essence what it was: "A guide to collectors,...aiming at utility rather than authority." Some of the changes introduced will not be popular. For example, though *Sorbus* is separated from *Pyrus*, yet *Pyrus* still contains the "Medlar." The work is remarkably free from typographical errors. It is a pity that *Scorzonera humilis*, which many will be surprised to see regarded as a British plant, has strayed into *Campanulaceae* by mistake. For nomenclature the Vienna Rules are not always followed; thus, the "Wych Elm" is called *Ulmus montana* Stokes instead of *U. glabra* Hudson, which is a name 25 years older than *U. montana* Stokes; and in the heading of the index the archaic and misleading term "Natural Order" is used.

On p. 4, H. C. Watson is quoted as saying that in several instances in the 7th edition of the Catalogue the census "number has been given too high through the impossibility of clearly distinguishing the truly native habitats from others improperly so reported." Though this is a real difficulty which still exists, we think the time has arrived to make some serious effort to discriminate between the records of trees from counties where they are probably native and those from counties where they are certainly planted. The Beech, for instance, has a census number of 67, probably twice as high as it should be. The Hornbeam (27) is nearer the mark, but is still somewhat too high. Miller Christy (*Journ. Ecol.* 12, 1924), after very careful consideration of available data, makes it 21 or 22, and this must be very close to the truth. The number for *Quercus robur* (105) is probably too high, and no census number is given for *Q. sessiliflora*, though surely there must now be enough trustworthy records to enable an approximately accurate number to be given it.

All into whose hands the catalogue falls will criticise it in one way or another; but all will find it a pleasant, well-printed, compendious list, in which the comparative rarity or frequency of most British plants may be seen at a glance.

H. G.-C.

INGHAM COLLECTION OF MOSSES

(Communicated)

Botanologists will be interested to learn that the late William Ingham's collection of British Mosses and Liverworts has, by gift, found a permanent home in the University of Leeds. It contains twelve thousand specimens, including gatherings by many well known men of the past, such as Prof. T. Barker, R. Barnes, Dr R. Braithwaite, T. Boswell, J. Needham, J. Nowell, Dr H. F. Parsons, W. H. Pearson, M. B. Slater, Dr R. Spruce, W. West, J. A. Wheldon and W. Wilson.

There is a large series of Harpidioid Hypna vouched by H. N. Dixon, Wheldon and Renauld that will be valuable for the study of that polymorphic group.

A comprehensive collection of *Sphagna* tested by Wheldon will supply types of the varieties and forms described in his synopsis of the European *Sphagna*.

Amongst plants having a sentimental value may be named the first British gatherings of *Tortula cernua* at Aberford and Conisborough; the first Yorkshire gathering of *Jubula Hutchinsiae* at Hebden Bridge; *Thuidium Blandovii* gathered by Barnes at Halnaby, probably now lost to the county by drainage of the Carrs. The known history of *Tetraplodon Wormskjoldii* in the country is illustrated by a number of gatherings, including Slaters plant (1370), which remained unrecognised until Jones and Horrell refound it in the same district in 1901.

Many vouchers for new county records were sent to Ingham as compiler of the Census Catalogues of British Mosses and Hepatics; it is to be hoped that these will attract similar material in the future. Leeds is accessible; a convenient centre for the West Riding or even for the north of England.

Mr W. H. Burrell, F.L.S., who has already expended time and skill in the arrangement of the specimens, has been appointed Honorary Curator of the Collection.

Vouchers for new plants or new county records, addressed to the Curator, will be placed in the collection.

October 27th, 1925.

